JC05 Rec'd PCT/PTO 26 MAR 2002

FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER
PI3750USW

U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR
10/089198

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/09639	**3 October 2000**	**5 October 1999**

TITLE OF INVENTION
CHEMICAL CONSTRUCTS

APPLICANT(S) FOR DO/EO/US
Robin Arthur Ellis CARR; Sylvie GEHANNE; Alfredo PAIO; Geoffrey Martyn WILLIAMS; Alessio ZARAMELLA

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

PCT publication cover page
copy of PCT Request
copies of Notification of the Recording of a Change (2)

Express Mail Label No.
EV022025843US

JC13 Rec'd PCT/PTO 26 MAR 2002

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
10/089198	PCT/EP00/09639	PI3750USW

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

	CALCULATIONS	PTO USE ONLY
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**		
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$890.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	64 - 20 =	44	x $18.00	**$792.00**	
Independent claims	4 - 3 =	1	x $84.00	**$84.00**	
Multiple Dependent Claims **(check if applicable).**			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,766.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$0.00**	
SUBTOTAL =				**$1,766.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$1,766.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$1,766.00**	
				Amount to be: refunded	$
				charged	$

a. ☐ A check in the amount of ______________ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. **07-1392** in the amount of **$1,766.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **07-1392** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

23347
PATENT TRADEMARK OFFICE

F. P. Grassler
SIGNATURE

Frank P. Grassler
NAME

31,164
REGISTRATION NUMBER

March 26, 2002
DATE

10089198 .072902

CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10) Applicant(s): **Carr et al.**			Docket No. **PI3750USW**
Serial No. **10/089,198**	Filing Date	Examiner	Group Art Unit

Invention: **CHEMICAL COMPOUNDS**

I hereby certify that the following correspondence:

Response to Notification of Missing Requirements under 35 USC 371

(Identify type of correspondence)

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 in an envelope addressed to: The Assistant Commissioner for Patents, Washington, D.C. 20231 on

July 29, 2002
(Date)

Marilyn Eldridge
(Typed or Printed Name of Person Mailing Correspondence)

Marilyn Eldridge
(Signature of Person Mailing Correspondence)

EL395893689US

Note: Each paper must have its own certificate of mailing.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	CARR, et al
International Application No.:	PCT/EP00/09639
International Filing Date:	October 3, 2000
Title:	***CHEMICAL CONSTRUCTS***

Commissioner for Patents
Washington, D.C. 20231

Attention: Box PCT/DO/EO/US

FIRST PRELIMINARY AMENDMENT

Sir:

The above identified application is being transmitted herewith for entry into the U.S. National Phase under Chapter II of the PCT. For the purposes of adding the priority information, please amend the application as follows:

In the Abstract:

Please substitute the attached Abstract, which has been placed on a separate piece of paper according to US practice.

In the Specification:

On the first line of the specification, after the Title, please add:

--This application is filed pursuant to 35 U.S.C. § 371 as a United States National Phase Application of International Application No. PCT/EP00/09639 filed October 3, 2000, which claims priority from GB9923577.2 filed October 5, 1999 –

Express Mail Label No.
EV022025843US

Clean Copy of Pending Claims

In the Claims:

1. A method of analysis of a solid phase construct; which method comprises:

(i) providing a chemical construct comprising a solid support Q having linked thereto via a connecting group Y a substrate R; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectrophotometry;

(ii) cleaving the connecting group Y at the first cleavage site to release the fragment Fr^u ; and

(iii) subjecting the fragment Fr^u to ultra violet, visible or fluorescence spectrophotometric analysis to quantify the substrate R.

2. A method according to claim 1 wherein the chromophore C^u has a principal log E_{max} value of at least 2.5.

3. A method according to claim 2 wherein the principal log E_{max} value is at least 1.5 times greater than the principal log E_{max} of the substrate R.

4. (Amended) A method according to claim 2 wherein the chromophore C^u has a principal log E_{max} that is at least 2 times greater than the principal log E_{max} of the substrate R.

5. (Amended) A method according to claim 1 wherein the chromophore C^u has an absorption band which is remote from any significant absorption bands due to the substrate R..

6. (Amended) A method according to claim 1 wherein the spectrophometric analysis is carried out at a wavelength corresponding to the principal E_{max} of the chromophore.

7. (Amended) A method according to claim 1 wherein the spectrophotometric analysis is carried out a wavelength corresponding to a non-principal absorption band of the chromophore.

8. A chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto via a connecting group Y a substrate R; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectroscopy, the chromophore C^u having a principal log E_{max} value of at least 2.5 and wherein (i) the principal log E_{max} value is at least 1.5 times greater than the principal log E_{max} of the

substrate R; or (ii) , the chromophore C^u has an absorption peak at a wavelength remote from absorptions due to the substrate R..

9. (Amended) A chemical construct according to claim 8 wherein the chromophore C^u has a principal log E_{max} which is at least 2 times greater than the principal log E_{max} of the substrate R.

10. A chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto via a connecting group Y a substrate R; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectroscopy, wherein the absorption characteristics of the chromophore C^u and the substrate R are such that at a given measurement wavelength, any errors in measurement of the quantity of substrate R (or any fragment or construct containing the fragment) arising from any overlap between absorption bands due to the chromophore and absorption bands due to the substrate R are less than 5%.

11. (Amended) A chemical construct according to claim 8 wherein the chromophore is a group containing a fused polycyclic aryl group.

12. (Amended) A chemical construct according to claim 11 wherein the polycylic aryl group is a C_6 - C_{30} polycyclic aryl group in which one or more

(e.g. 1, 2, or 3) ring carbon atoms are optionally replaced by a heteroatom such as nitrogen, sulphur or oxygen.

13. A chemical construct according to claim 12 wherein the polycyclic aryl group is selected from polycyclic hydrocarbons such as naphthyl, phenanthrenyl and anthracenyl groups, and polycyclic heteroaryl groups such as acridine, or phenanthroline.

14. A chemical construct according to claim 13 wherein the chromophore contains an anthracenyl group.

15. A chemical construct according to claim 12 wherein the chromophore contains a dansyl (5-dimethylamino-1-naphthylsulphonyl) group.

16. (Amended) A chemical construct according to claim 8 wherein the fragment Fr^u contains a mass spectroscopic sensitising group G.

17. (Amended) A chemical construct according to claim 8 wherein the chemical fragment Fr^u contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

18. A chemical construct according to claim 16 wherein the means for imparting a characteristic signature to the mass spectrum of the fragment Fr^u is located between the first and second cleavage sites.

19. (Amended) A chemical construct according to claim 8 wherein the first and second cleavage sites are defined by first and second linker groups L^1 and L^2.

20. A chemical construct according to claim 19 wherein a spacer group A is interposed between the two linker groups L^1 and L^2, the spacer group A containing means for imparting a characteristic signature to the mass spectrum of the fragment Fr^u.

21. (Amended) A chemical construct according to claim 8 containing a sensitising group G which is a group which is ionisable under mass spectrometric conditions.

22. A chemical construct according to claim 21 wherein the group G is ionisable to form a positive ion under mass spectrometric conditions, for example electrospray mass spectrometric conditions.

23. A method of analysis of a solid phase construct; which method comprises:

(i) providing a chemical construct comprising a solid support Q having linked thereto via a connecting group Y a substrate R, the substrate R being present on each solid support in an amount of no more than 10 nanomoles; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectrophotometry;

(ii) isolating a solid support, or a plurality of solid supports not exceeding 20 in number;

(ii) treating the solid support(s) to cleave the connecting group at the first cleavage site to release the fragment Fr^u containing the substrate R; and

(Iii) subjecting the fragment Fr^u to ultra violet, visible or fluorescence spectrophotometric analysis to quantify the substrate R.

24. A method according to claim 23 wherein the solid support Q is a resin bead having an average diameter in the range from 90μm to 250μm.

25. (Amended) A method according to claim 23 wherein the substrate R is present on each solid support in an amount of less than 5 nanomoles.

26. A method according to claim 25 wherein the substrate R is present on each solid support in an amount of less than 2 nanomoles.

27. (Amended) A method according to claim 23 wherein the number of solid supports isolated is less than 10, for example less than 5.

28. A method according to claim 27 wherein a single solid support is isolated and subjected to cleavage.

29. Canceled.

30. (Amended) A method of identifying a pharmaceutically useful substrate comprising preparing a library containing a plurality of chemical constructs as defined in claim 8, and subjecting the library to biological testing to identify biologically active substrates.

31. A method according to claim 30 which includes the further step of formulating a biologically active substrate thus identified with a pharmaceutically acceptable carrier to form a pharmaceutical composition.

32. (Amended) An intermediate chemical construct for use preparing a chemical construct as defined in claim 8, the intermediate construct having the formula Q-Y' wherein Y' is a reactive or protected form of the group Y.

33. (Amended) An intermediate construct of the formula $Q\text{-}L^1\text{-}A^p$ wherein Q and L^1 are as defined in claim 8 and A^p is a reactive or protected form of the spacer group A containing a chromophore C^u and optionally a peak splitting isotopic label.

34. An intermediate construct according to claim 33 having the general formula $Q\text{-}L^1\text{-}N(Alk - C^u)\text{-}Alk\text{-}NH\text{-}X^1$ wherein Alk is an alkylene group and X^1 is hydrogen or an aralkyl group.

Please add the following new claims.

35. (New) A chemical construct according to claim 10 wherein the chromophore is a group containing a fused polycyclic aryl group.

36. (New) A chemical construct according to claim 35 wherein the polycylic aryl group is a C_6 - C_{30} polycyclic aryl group in which one or more (e.g. 1, 2, or 3) ring carbon atoms are optionally replaced by a heteroatom such as nitrogen, sulphur or oxygen.

37. (New) A chemical construct according to claim 36 wherein the polycyclic aryl group is selected from polycyclic hydrocarbons such as naphthyl, phenanthrenyl and anthracenyl groups, and polycyclic heteroaryl groups such as acridine, or phenanthroline.

38. (New) A chemical construct according to claim 37 wherein the chromophore contains an anthracenyl group.

39. (New) A chemical construct according to claim 36 wherein the chromophore contains a dansyl (5-dimethylamino-1-naphthylsulphonyl) group.

40. A chemical construct according to claim 10 wherein the fragment Fr^u contains a mass spectroscopic sensitising group G.

41. (New) A chemical construct according to claim 10 wherein the chemical fragment Fr^u contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

42. (New) A chemical construct according to claim 40 wherein the means for imparting a characteristic signature to the mass spectrum of the fragment Fr^u is located between the first and second cleavage sites.

43. A chemical construct according to claim 10 wherein the first and second cleavage sites are defined by first and second linker groups L^1 and L^2.

44. (New) A chemical construct according to claim 43 wherein a spacer group A is interposed between the two linker groups L^1 and L^2, the

spacer group A containing means for imparting a characteristic signature to the mass spectrum of the fragment Fr^u .

45. (New) A chemical construct according to claim 10 containing a sensitising group G which is a group which is ionisable under mass spectrometric conditions.

46. (New) A method of identifying a pharmaceutically useful substrate comprising preparing a library containing a plurality of chemical constructs as defined in claim 10, and subjecting the library to biological testing to identify biologically active substrates.

47. (New) A method according to claim 46 which includes the further step of formulating a biologically active substrate thus identified with a pharmaceutically acceptable carrier to form a pharmaceutical composition.

48. (New) An intermediate chemical construct for use preparing a chemical construct as defined in claim 10, the intermediate construct having the formula Q-Y' wherein Y' is a reactive or protected form of the group Y.

49. (New) An intermediate construct of the formula $Q\text{-}L^1\text{-}A^p$ wherein Q and L^1 are as defined in claim 10 and A^p is a reactive or protected form of the spacer group A containing a chromophore C^u and optionally a peak splitting isotopic label.

50. (New) An intermediate construct according to claim 49 having the general formula $Q\text{-}L^1\text{-}N(Alk - C^u)\text{-}Alk\text{-}NH\text{-}X^1$ wherein Alk is an alkylene group and X^1 is hydrogen or an aralkyl group.

51. A method according to claim 23 wherein the chromophore C^u has a principal log E_{max} value of at least 2.5 and wherein (i) the principal log E_{max} value is at least 1.5 times greater than the principal log E_{max} of the substrate R; or (ii) , the chromophore C^u has an absorption peak at a wavelength remote from absorptions due to the substrate R.

52. (New) A method according to claim 23 wherein the chromophore C^u has a principal log E_{max} which is at least 2 times greater than the principal log E_{max} of the substrate R.

53. (New) A method according to claim 23, wherein the absorption characteristics of the chromophore C^u and the substrate R are such that at a given measurement wavelength, any errors in measurement of the quantity of substrate R (or any fragment or construct containing the fragment) arising from any overlap between absorption bands due to the chromophore and absorption bands due to the substrate R are less than 5%.

54. (New) A method according to claim 23 wherein the chromophore is a group containing a fused polycyclic aryl group.

55. (New) A method according to claim 54 wherein the polycylic aryl group is a C_6 - C_{30} polycyclic aryl group in which one or more (e.g. 1, 2, or 3) ring carbon atoms are optionally replaced by a heteroatom such as nitrogen, sulphur or oxygen.

56. (New) A method according to claim 55 wherein the polycyclic aryl group is selected from polycyclic hydrocarbons such as naphthyl, phenanthrenyl and anthracenyl groups, and polycyclic heteroaryl groups such as acridine, or phenanthroline.

57. (New) A method according to claim 56 wherein the chromophore contains an anthracenyl group.

58. (New) A method according to claim 55 wherein the chromophore contains a dansyl (5-dimethylamino-1-naphthylsulphonyl) group.

59. (New) A method according to claim 23 wherein the fragment Fr^u contains a mass spectroscopic sensitising group G.

60. (New) A method according to claim 23 wherein the chemical fragment Fr^u contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

61. (New) A method according to claim 59 wherein the means for imparting a characteristic signature to the mass spectrum of the fragment Fr^u is located between the first and second cleavage sites.

62. (New) A method according to claim 23 wherein the first and second cleavage sites are defined by first and second linker groups L^1 and L^2.

63. (New) A method according to claim 62 wherein a spacer group A is interposed between the two linker groups L^1 and L^2, the spacer group A containing means for imparting a characteristic signature to the mass spectrum of the fragment Fr^u .

64. (New) A method according to claim 23 containing a sensitising group G which is a group which is ionisable under mass spectrometric conditions.

65. (New) A method according to claim 64 wherein the group G is ionisable to form a positive ion under mass spectrometric conditions, for example electrospray mass spectrometric conditions.

REMARKS

Currently claims 1-65 are pending. Claim 29 has been canceled, claims 4-7, 9, 11-12, 16-17, 19, 21, 25, 27, 30, and 32-33 have been amended to place them in form appropriate to US practice and to reduce the filing fee by removing multiple dependency, and new claims 35-65 have been added. Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned **"Version With Markings To Show Changes Made".** Applicants have attached an abstract on a separate sheet of paper as required by US practice. Applicants have amended the specification for purposes of adding the priority information.

Respectfully submitted,

Frank P. Grassler March 26, 2002

Frank P. Grassler
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Date: March 26, 2002
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CHEMICAL CONSTRUCTS

Abstract

A chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto via a connecting group Y a substrate R; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectroscopy, the chromophore C^u having a principal log E_{max} value of at least 2.5 and wherein (i) the principal log E_{max} value is at least 1.5 times greater than the principal log E_{max} of the substrate R; or (ii) , the chromophore C^u has an absorption peak at a wavelength remote from absorptions due to the substrate R; and to methods of analysis of products of solid phase synthesis using the constructs.

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Marked-Up Copy of Pending Claims

In the Claims:

1. A method of analysis of a solid phase construct; which method comprises:

(i) providing a chemical construct comprising a solid support Q having linked thereto via a connecting group Y a substrate R; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectrophotometry;

(ii) cleaving the connecting group Y at the first cleavage site to release the fragment Fr^u ; and

(iii) subjecting the fragment Fr^u to ultra violet, visible or fluorescence spectrophotometric analysis to quantify the substrate R.

2. A method according to claim 1 wherein the chromophore C^u has a principal log E_{max} value of at least 2.5.

3. A method according to claim 2 wherein the principal log E_{max} value is at least 1.5 times greater than the principal log E_{max} of the substrate R.

4. (Amended) A method according to claim 2 [or claim 3] wherein the chromophore C^u has a principal log E_{max} that is at least 2 times greater than the principal log E_{max} of the substrate R.

5. (Amended) A method according to [any one of the preceding claims] claim 1 wherein the chromophore C^u has an absorption band which is remote from any significant absorption bands due to the substrate R..

6. (Amended) A method according to [any one of the preceding claims] claim 1 wherein the spectrophometric analysis is carried out at a wavelength corresponding to the principal E_{max} of the chromophore.

7. (Amended) A method according to [any one of claims 1 to 5] claim 1 wherein the spectrophotometric analysis is carried out a wavelength corresponding to a non-principal absorption band of the chromophore.

8. A chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto via a connecting group Y a substrate R; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectroscopy, the chromophore C^u having a principal log E_{max} value of at least 2.5 and wherein (i) the principal log E_{max} value is at least 1.5 times greater than the principal log E_{max} of the

substrate R; or (ii) , the chromophore C^u has an absorption peak at a wavelength remote from absorptions due to the substrate R..

9. (Amended) A chemical construct according to claim [7] 8 wherein the chromophore C^u has a principal log E_{max} which is at least 2 times greater than the principal log E_{max} of the substrate R.

10. A chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto via a connecting group Y a substrate R; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectroscopy, wherein the absorption characteristics of the chromophore C^u and the substrate R are such that at a given measurement wavelength, any errors in measurement of the quantity of substrate R (or any fragment or construct containing the fragment) arising from any overlap between absorption bands due to the chromophore and absorption bands due to the substrate R are less than 5%.

11. (Amended) A chemical construct according to [any one of claims 8 to 10] claim 8 wherein the chromophore is a group containing a fused polycyclic aryl group.

12. (Amended) A chemical construct according to claim 11 wherein the polycylic aryl group is a C_6 - C_{30} polycyclic aryl group in which one or more

(e.g. 1, 2, or 3) ring carbon atoms are optionally replaced by a heteroatom such as nitrogen, sulphur or oxygen.

13. A chemical construct according to claim 12 wherein the polycyclic aryl group is selected from polycyclic hydrocarbons such as naphthyl, phenanthrenyl and anthracenyl groups, and polycyclic heteroaryl groups such as acridine, or phenanthroline.

14. A chemical construct according to claim 13 wherein the chromophore contains an anthracenyl group.

15. A chemical construct according to claim 12 wherein the chromophore contains a dansyl (5-dimethylamino-1-naphthylsulphonyl) group.

16. (Amended) A chemical construct according to [any one of claims 8 to 15] <u>claim 8</u> wherein the fragment Fr^u contains a mass spectroscopic sensitising group G.

17. (Amended) A chemical construct according to [any one of claims 8 to 16] <u>claim 8</u> wherein the chemical fragment Fr^u contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

18. A chemical construct according to claim 16 wherein the means for imparting a characteristic signature to the mass spectrum of the fragment Fr^u is located between the first and second cleavage sites.

19. (Amended) A chemical construct according to [any one of claims 8 to 18] claim 8 wherein the first and second cleavage sites are defined by first and second linker groups L^1 and L^2.

20. A chemical construct according to claim 19 wherein a spacer group A is interposed between the two linker groups L^1 and L^2, the spacer group A containing means for imparting a characteristic signature to the mass spectrum of the fragment Fr^u.

21. (Amended) A chemical construct according to [any one of claims 8 to 20] claim 8 containing a sensitising group G which is a group which is ionisable under mass spectrometric conditions.

22. A chemical construct according to claim 21 wherein the group G is ionisable to form a positive ion under mass spectrometric conditions, for example electrospray mass spectrometric conditions.

23. A method of analysis of a solid phase construct; which method comprises:

(i) providing a chemical construct comprising a solid support Q having linked thereto via a connecting group Y a substrate R, the substrate R being present on each solid support in an amount of no more than 10 nanomoles; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the

said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectrophotometry;

(ii) isolating a solid support, or a plurality of solid supports not exceeding 20 in number;

(ii) treating the solid support(s) to cleave the connecting group at the first cleavage site to release the fragment Fr^u containing the substrate R; and

(Iii) subjecting the fragment Fr^u to ultra violet, visible or fluorescence spectrophotometric analysis to quantify the substrate R.

24. A method according to claim 23 wherein the solid support Q is a resin bead having an average diameter in the range from 90μm to 250μm.

25. (Amended) A method according to claim 23 [or claim 24] wherein the substrate R is present on each solid support in an amount of less than 5 nanomoles.

26. A method according to claim 25 wherein the substrate R is present on each solid support in an amount of less than 2 nanomoles.

27. (Amended) A method according to [any one of claims 23 to 26] claim 23 wherein the number of solid supports isolated is less than 10, for example less than 5.

28. A method according to claim 27 wherein a single solid support is isolated and subjected to cleavage.

29. Canceled.

30. (Amended) A method of identifying a pharmaceutically useful substrate comprising preparing a library containing a plurality of chemical constructs as defined in [any one of claims 8 to 22] claim 8, and subjecting the library to biological testing to identify biologically active substrates.

31. A method according to claim 30 which includes the further step of formulating a biologically active substrate thus identified with a pharmaceutically acceptable carrier to form a pharmaceutical composition.

32. (Amended) An intermediate chemical construct for use preparing a chemical construct as defined in [any one of claims 8 to 22] claim 8, the intermediate construct having the formula Q-Y' wherein Y' is a reactive or protected form of the group Y.

33. (Amended) An intermediate construct of the formula $Q\text{-}L^1\text{-}A^p$ wherein Q and L^1 are as defined in [any one of claims 8 to 22] claim 8 and A^p is a reactive or protected form of the spacer group A containing a chromophore C^u and optionally a peak splitting isotopic label.

34. An intermediate construct according to claim 33 having the general formula $Q\text{-}L^1\text{-}N(Alk - C^u)\text{-}Alk\text{-}NH\text{-}X^1$ wherein Alk is an alkylene group and X^1 is hydrogen or an aralkyl group.

Please add the following new claims:

--35. (New) A chemical construct according to claim 10 wherein the chromophore is a group containing a fused polycyclic aryl group.

36. (New) A chemical construct according to claim 35 wherein the polycylic aryl group is a C_6 - C_{30} polycyclic aryl group in which one or more (e.g. 1, 2, or 3) ring carbon atoms are optionally replaced by a heteroatom such as nitrogen, sulphur or oxygen.

37. (New) A chemical construct according to claim 36 wherein the polycyclic aryl group is selected from polycyclic hydrocarbons such as naphthyl, phenanthrenyl and anthracenyl groups, and polycyclic heteroaryl groups such as acridine, or phenanthroline.

38. (New) A chemical construct according to claim 37 wherein the chromophore contains an anthracenyl group.

39. (New) A chemical construct according to claim 36 wherein the chromophore contains a dansyl (5-dimethylamino-1-naphthylsulphonyl) group.

40. (New) A chemical construct according to claim 10 wherein the fragment Fr^u contains a mass spectroscopic sensitising group G.

41. (New) A chemical construct according to claim 10 wherein the chemical fragment Fr^u contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

42. (New) A chemical construct according to claim 40 wherein the means for imparting a characteristic signature to the mass spectrum of the fragment Fr^u is located between the first and second cleavage sites.

43. (New) A chemical construct according to claim 10 wherein the first and second cleavage sites are defined by first and second linker groups L^1 and L^2.

44. (New) A chemical construct according to claim 43 wherein a spacer group A is interposed between the two linker groups L^1 and L^2, the spacer group A containing means for imparting a characteristic signature to the mass spectrum of the fragment Fr^u .

45. (New) A chemical construct according to claim 10 containing a sensitising group G which is a group which is ionisable under mass spectrometric conditions.

46. (New) A method of identifying a pharmaceutically useful substrate comprising preparing a library containing a plurality of chemical constructs as defined in claim 10, and subjecting the library to biological testing to identify biologically active substrates.

47. (New) A method according to claim 46 which includes the further step of formulating a biologically active substrate thus identified with a pharmaceutically acceptable carrier to form a pharmaceutical composition.

48. (New) An intermediate chemical construct for use preparing a chemical construct as defined in claim 10, the intermediate construct having the formula Q-Y' wherein Y' is a reactive or protected form of the group Y.

49. (New) An intermediate construct of the formula $Q\text{-}L^1\text{-}A^p$ wherein Q and L^1 are as defined in claim 10 and A^p is a reactive or protected form of the

spacer group A containing a chromophore C^u and optionally a peak splitting isotopic label.

50. (New) An intermediate construct according to claim 49 having the general formula $Q\text{-}L^1\text{-}N(Alk - C^u)\text{-}Alk\text{-}NH\text{-}X^1$ wherein Alk is an alkylene group and X^1 is hydrogen or an aralkyl group.

51. (New) A method according to claim 23 wherein the chromophore C^u has a principal log E_{max} value of at least 2.5 and wherein (i) the principal log E_{max} value is at least 1.5 times greater than the principal log E_{max} of the substrate R; or (ii) , the chromophore C^u has an absorption peak at a wavelength remote from absorptions due to the substrate R.

52. (New) A method according to claim 23 wherein the chromophore C^u has a principal log E_{max} which is at least 2 times greater than the principal log E_{max} of the substrate R.

53. (New) A method according to claim 23, wherein the absorption characteristics of the chromophore C^u and the substrate R are such that at a given measurement wavelength, any errors in measurement of the quantity of substrate R (or any fragment or construct containing the fragment) arising from any overlap between absorption bands due to the chromophore and absorption bands due to the substrate R are less than 5%.

54. (New) A method according to claim 23 wherein the chromophore is a group containing a fused polycyclic aryl group.

55. (New) A method according to claim 54 wherein the polycylic aryl group is a C_6 - C_{30} polycyclic aryl group in which one or more (e.g. 1, 2, or 3)

ring carbon atoms are optionally replaced by a heteroatom such as nitrogen, sulphur or oxygen.

56. (New) A method according to claim 55 wherein the polycyclic aryl group is selected from polycyclic hydrocarbons such as naphthyl, phenanthrenyl and anthracenyl groups, and polycyclic heteroaryl groups such as acridine, or phenanthroline.

57. (New) A method according to claim 56 wherein the chromophore contains an anthracenyl group.

58. (New) A method according to claim 55 wherein the chromophore contains a dansyl (5-dimethylamino-1-naphthylsulphonyl) group.

59. (New) A method according to claim 23 wherein the fragment Fr^u contains a mass spectroscopic sensitising group G.

60. (New) A method according to claim 23 wherein the chemical fragment Fr^u contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

61. (New) A method according to claim 59 wherein the means for imparting a characteristic signature to the mass spectrum of the fragment Fr^u is located between the first and second cleavage sites.

62. (New) A method according to claim 23 wherein the first and second cleavage sites are defined by first and second linker groups L^1 and L^2.

63. (New) A method according to claim 62 wherein a spacer group A is interposed between the two linker groups L^1 and L^2, the spacer group A containing means for imparting a characteristic signature to the mass spectrum of the fragment Fr^u

64. (New) A method according to claim 23 containing a sensitising group G which is a group which is ionisable under mass spectrometric conditions.

65. (New) A method according to claim 64 wherein the group G is ionisable to form a positive ion under mass spectrometric conditions, for example electrospray mass spectrometric conditions.--

CHEMICAL CONSTRUCTS

Field of the Invention

This invention relates to chemical constructs for use in solid phase synthesis, and to methods of analysis of the products of solid phase synthesis using the constructs.

Background of the Invention

Solid phase synthesis has been known for many years in the field of peptide synthesis and more recently has also been used increasingly for the synthesis of non-peptides.

Solid phase synthesis has found particular application in the field of combinatorial chemistry and the preparation of chemical libraries as potential sources of new leads for drug discovery, see for example Anthony W. Czarnik, *Analytical Chemistry News and Features*, June 1, 1998, pp 378A-386A, and *The Combinatorial Index*, Barry A. Bunin, Academic Press, San Diego 1998. A feature of combinatorial chemistry methods is that they enable very large numbers of different compounds to be prepared from a relatively limited number of molecular building blocks in a relatively small number of reactions. Combinatorial chemistry makes use of the "split and pool" approach in which a suspension of chemical starting material tethered to a solid support is split into N portions, each of which is reacted with a different reagent. The products of the N reactions are then pooled and mixed thoroughly, the resulting pool is split into N' portions and again each portion is reacted with a different reagent. This procedure can be repeated as many times as there are steps in the reaction sequence. Thus, for a three step reaction sequence, if the reaction mixture is divided into ten portions at each stage, each of the pools being reacted with a different reagent before recombining with the other portions, the total number of compounds formed by the process will be $10^3 = 1000$. Thus it can be seen that by using the split and pool technique, a large number of different molecules can be synthesised using a minimal number of reactions (thirty in the case mentioned above).

Each individual solid support (e.g. a resin bead) will have a single product molecule tethered to it and hence, in principle, each of the reaction products can be separated and

analysed or subjected to biological testing simply by isolating each single solid support and cleaving the product from the support. However, the large numbers of compounds generated by combinatorial methods means that it can be impracticable to identify and characterise each compound. Consequently, the compounds usually are first tested, either on the solid support or after cleaving from the support, and only those compounds, which show some biological activity, are subsequently identified. In order to minimise the number of biological tests carried out, compounds can be tested in pools containing a predetermined number of compounds, the inactive pools being discarded and the active pools being subject to further investigation. The biological activities of the compounds can be analysed using high throughput automated assay techniques permitting large numbers of compounds to be analysed in a short time.

One of the problems facing the chemist working with solid phase constructs is how to identify and characterise the various compounds in a combinatorial library since each compound will be present in the library in very small amounts, and there will usually be insufficient compound present on a given solid support to allow for both biological testing and identification of the compound. The resin beads typically used in the synthesis of combinatorial libraries are derived from cross-linked polystyrene resin and are usually of the order of 90μm and 250μm in diameter (i.e. just visible to the naked eye). The number of beads in a given mass or volume of resin will depend on the average bead size but, for example, with a bead size of 150μm diameter, there will be approximately 500 beads per milligram of bulk resin. Typical compound loadings on the beads are of the order of 0.1 to 0.4 mmol g^{-1} and hence beads of the dimensions given above will tend to have individual compound loadings of about 1 nanomole of compound per bead.

One known approach to the problem of how to identify the compounds in a combinatorial library is to provide each solid support with a coding tag from which the identity of the compound can be determined. Thus, for example, a coding tag can be built up in sequential steps on the solid support in parallel with the construction of the desired target compound, the coding tag reflecting the synthesis history of the product compound and being unique for each product compound. The coding tag is usually built up on the support using chemical reactions of a type which are orthogonal to the chemistry used to build up the product compound, thereby ensuring that the coding units and product compounds do not become confused. Once the product compound has been tested, and its biological activity confirmed, the coding tag can then be decoded to allow identification of the compound.

In an alternative approach, as described in Geysen *et al.*, Chemistry & Biology, 1996, Vol. 3, No. 8, pp 679-688, and in WO 97/37953, coding tags such as isotopic labels can be incorporated into *inter alia* a linker group between the solid support and the substrate. This approach has the advantage that it does not require the formation of a separate coding tag using orthogonal chemistry and thereby reduces the overall number of synthesis steps involved.

A further problem facing the solid phase chemist is the difficulty in quantifying the products of a given reaction sequence. In any solid phase synthesis procedure, whether or not part of a combinatorial procedure, it is important to be able to determine the optimal conditions for a given reaction step in a series of steps. It is also important to be able to monitor reactions, for example so that it can be determined whether a particular reaction has gone to completion. This is particularly important in a multistage solid phase synthesis where the failure of a given stage to proceed to completion can lead to the formation of side products thereby complicating what is otherwise a relatively straightforward separation procedure. Where multiple products are formed in a given reaction step or sequence of steps, or where a given reaction step has not proceeded to completion, it can be extremely useful to have a means of quantifying the reaction products, either in absolute terms, or in terms of the relative concentrations with respect to other reaction products. Where the products are produced in milligram quantities, quantitation can be carried out using a variety of instrumental techniques, for example nuclear magnetic resonance (nmr) spectroscopy. However, quantitation is much more difficult where the amount of product available for analysis may be as little as 1 nanomole.

Although both destructive and non-destructive analytical methods are known for determining the products of solid phase synthesis (see *The Combinatorial Index, idem*), one of the recognised problems with solid phase synthesis is that it is generally more difficult to monitor reactions than it is to monitor conventional solution-phase reactions; see for example the Czarnik paper referred to above. This is particularly true with the products of combinatorial chemistry methods where there is a need to use rapid high throughput techniques to analyse the large numbers of compounds generated by such methods. Techniques such as mass spectrometry are potentially ideally suited as a means of providing high throughput analyses, but a substantial problem is that not all compounds produce a guaranteed response under mass spectrometric conditions. Indeed, many compounds are "invisible" to the so-called "soft" methods of mass spectrometry such as Matrix-Assisted Laser-Desorption Ionisation (MALDI) and Electrospray mass spectrometry which are intended to detect molecular ions

without causing significant fragmentation of the molecule. In this context, the term "soft " means mass spectral methods that give molecular ions and not merely fragments. One of the reasons for this is that under MALDI and electrospray conditions, many compounds, particularly peptides, do not ionise sufficiently well to give a detectable mass spectral response.

Geysen et al. (idem) address this problem by suggesting the introduction of a readily ionisable basic centre such as lysine into the linker, and further suggest that the basic centre could be quaternised by alkylation during work up in order to give a charged group which would sensitise the construct to mass spectrometric analysis. However, one drawback of this approach is the need to protect the amine group during the various synthesis steps and the consequent need to deprotect the amine group prior to analysis.

A development of the Geysen approach is disclosed in Carrasco *et al. Tetrahedron Letters*, 1997, 38, No. 36, pp 6331-6334. Carrasco's method involves the formation of a construct comprising a resin bead linked via a first linker group to a group, which the authors refer to as an "ionisation tag". The "ionisation tag" in turn is linked via a second linker group to a substrate. The first and second linker groups are orthogonally cleavable; i.e. they can be selectively cleaved using different chemistries. In the specific example disclosed in Carrasco *et al.*, the first linker group is photochemically cleavable whilst the second linker group is chemically cleavable. The "ionisation tag" used by Carrasco is a tetrapeptide chain having the sequence (reading from the C-terminus) Gly-Phe-Lys-Ala, and having an N-(2-trimethylammonium)-acetyl group linked to the lysine. The purpose of the ionisation tag is twofold. Firstly, it provides an already ionised "sensitiser group" which enables the construct to be detected by matrix-assisted laser-desorption ionisation (MALDI) mass spectrometry, and secondly, it adds mass to the construct thereby enabling substrate molecules to be detected without being swamped or masked by low molecular weight peaks in the mass spectrum.

As explained above, a further problem with solid phase syntheses, particularly combinatorial syntheses, or other reaction sequences in which relatively small amounts of product are formed, concerns the difficulty in accurately quantifying the products of the reaction, particularly when the amounts of product are as little as1 nanomole. Although mass spectrometry is highly suitable for identifying the products of a reaction, it is not generally suitable as a means of measuring either absolute concentrations of products, or the relative concentrations of products in a mixture.

Summary of the Invention

It is an object of the present invention to provide a means of monitoring chemical reactions on solid supports which avoids problems inherent in known methods and which provides a means of quantitatively analysing the products of solid phase synthetic techniques. It is a further object of the invention to provide a means of quantitatively analysing the products of a solid phase synthesis where the amounts of product available for analysis are as little as 1 nanomole.

Accordingly, in a first aspect, the invention provides a method of analysis of a solid phase construct; which method comprises:

(i) providing a chemical construct comprising a solid support Q having linked thereto via a connecting group Y a substrate R; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectrophotometry;

(ii) cleaving the connecting group at the first cleavage site to release the fragment Fr^u ; and

(iii) subjecting the fragment Fr^u to ultra violet, visible or fluorescence spectrophotometric analysis to quantify the substrate R.

In accordance with the method of the invention, the spectrophotometric analysis may be used to determine either the absolute amount of substrate R present in a given sample, or it may be used to determine the relative amount (e.g. in terms of a molar ratio) of substrate R present relative to another component (e.g. where more than one substrate R is present) in the sample. References to methods of quantifying the substrate R in this application include both absolute determination and relative determination, unless the context indicates otherwise.

In order to ensure that any chromophores present in the substrate R do not interfere with the analysis, the chromophore is typically selected such that it has a substantial absorption band that distinguishes it from the substrate. Thus, for example, the absorption band may be remote from any significant absorption bands in the substrate. Alternatively, the absorption

band in the chromophore may be of such an intensity that it effectively swamps any overlapping band in the substrate. It is preferred that the chromophore C^u has a principal log E_{max} value of at least 2.5. It is further preferred that the principal log E_{max} value is at least 1.5 times greater than the principal log E_{max} of the substrate R.

Preferably the spectrophotometric analysis is carried out at a wavelength(s) in the ultraviolet region of the spectrum.

The relationship between the absorption of visible or UV radiation by a compound in solution and the concentration of the compound is defined by the Beer-Lambert law which can be expressed by the formula $E = A/cl$ where A is the absorbance or optical density of a sample solution, c is the molar concentration, l is the path length of the sample, and E is the molar absorptivity. The molar absorptivity E is constant for a given compound at a given wavelength and is usually expressed as E_{max} - the molar absorptivity at an absorption band maximum. The values for E or E_{max} are typically expressed in units of $10^{-2}\ m^2\ mol^{-1}$ and, unless indicated otherwise, references to E or E_{max} values herein will refer to such units. Since E or E_{max} values can be very large, the logarithmic equivalent is often used and such logarithmic equivalents are referred to herein as log E_{max} values.

A given compound will often have significant absorption peaks or bands at several different wavelengths and hence can often be defined with reference to several E_{max} values. In the context of the present invention, the term "principal E_{max} " is used to denote the E_{max} at the wavelength at which the greatest absorbance occurs. Thus, in the constructs of the present invention, the chromophore C^u preferably has a principle log E_{max} value of at least 2.5 and typically has a principal log E_{max} value which is at least 1.5 times greater than the principal E_{max} of the substrate R. More typically however, the chromophore C^u has a principal log E_{max} that is at least 2, more usually 2.5, and preferably 3 times greater than the principal log E_{max} of the substrate R.

Although analysis of the compounds can be effected by measuring absorbance at a principal maximum, measurement of side bands or lesser maxima may be used instead to determine the amount of compound present. In general, the wavelength at which the measurement is taken will depend on the precise absorption characteristics of the substrate R and the chromophore C^u, and can be determined on a case by case basis.

The chromophore C^u may be distinguished from any chromophores in the substrate in that it may possess an absorption band which is remote from any significant absorption bands. By this is meant that the absorption band of the chromophore is remote from, and does not overlap to any significant extent (e.g. less than 5% overlap with respect to the area under the curve (a.u.c.) of the respective absorption peaks) with any significant absorptions due to the substrate R. A significant absorption in the present context means an absorption having a log E value of 1 or more.

In a further aspect, the invention provides a chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto via a connecting group Y a substrate R; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectrophotometry (preferably ultraviolet spectrophotometry), the chromophore C^u having a principal log E_{max} value of at least 2.5 and wherein (i) the principal log E_{max} value is at least 1.5 times greater than the principal log E_{max} of the substrate R; or (ii) , the chromophore C^u has an absorption peak at a wavelength remote from absorptions due to the substrate R.

The presence of a UV chromophore in the construct enables quantitation of the products of a synthesis to be carried out. Such quantitation can be (i) absolute quantitation or (ii) relative quantitation, or both. By absolute quantitation is meant that the absolute concentrations of a substrate molecule or fragment or construct containing the substrate molecule can be determined, whereas the term " *relative quantitation* " is used herein to mean the determination of the amount of a substrate (or fragment or construct containing the substrate) relative to another component (such as a side product or starting material or another substrate) in a reaction mixture.

The UV chromophore is typically selected such that it has a very strong absorption at a unique or characteristic wavelength, which is usually distinct from the wavelengths at which the maximum absorbences of a typical substrate molecule might be found. However, in cases where the absorption of the chromophore is very large relative to the substrate or substrate R,

the overlapping of absorptions due to the substrate and the chromophore may have a minimal impact on the accuracy of the measurement of the quantity of substrate. In general, overlapping absorption bands should not prevent meaningful analyses to be carried out provided that any error introduced is less than about 10% and preferably no more than about 5%. Accordingly, as an alternative to defining the chromophore in terms of relative absorbances and absolute molar absorption values, the chromophore C, and its relationship with the absorption due to the substrate can be defined in terms of the degree of error arising from any overlap between the absorption bands of the substrate R and the chromophore.

Thus, in a further aspect, the invention provides a chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto via a connecting group Y a substrate R; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectroscopy, wherein the absorption characteristics of the chromophore C^u and the substrate R are such that at a given measurement wavelength, any errors in measurement of the quantity of substrate R (or any fragment or construct containing the fragment) arising from any overlap between absorption bands due to the chromophore and absorption bands due to the substrate R are less than 10% and preferably less than 5%.

Examples of chromophores are groups containing an aryl group, preferably a fused polycyclic aryl group, e.g. a C_6 - C_{30} polycyclic aryl group in which one or more (e.g. 1, 2, or 3) ring carbon atoms are optionally replaced by a heteroatom such as nitrogen, sulphur or oxygen. Examples of polycyclic aryl groups are polycyclic hydrocarbons such as naphthyl, phenanthrenyl and anthracenyl groups, and polycyclic heteroaryl groups such as acridine, or phenanthroline. Such aryl groups or polycyclic groups can be optionally substituted, preferably with a substituent or substituents which is or are inert and/or non-interfering with regard to the reaction conditions employed in a given reaction scheme. Examples of such substituent groups include alkyl and alkoxy (e.g. alkyl and alkoxy having from 1 to 20 carbon atoms, preferably from 1 to 6 carbons), each optionally containing one or more unsaturated linkages, and being optionally interrupted by one or more heteroatoms selected from oxygen, nitrogen and sulphur; amino (preferably protected amino or mono-or disubstituted amino, e.g.

dimethylamino); halogen such as fluoro, chloro, bromo and iodo; alkylthio (e.g. C_{1-20} alkylthio, preferably C_{1-6}), hydroxy, protected (e.g. acylated) hydroxy, thio, protected (e.g. acylated) thio, trifluoromethyl, nitro, alkylsulphonyl, aryl (e.g. optionally substituted phenyl, thienyl, furanyl), and arylalkyl (e.g. benzyl and phenethyl).

Other examples of chromophores include highly conjugated aryl or non-aryl systems, provided that such compounds are inert under the synthetic conditions employed in the solid phase chemistry, and so-called "push-pull" systems of the type G-J-M wherein G is an electron-donating group (e.g. amino or alkoxy), J is an conjugated array of multiple, e.g. double, bonds, and M is an electron withdrawing group (e.g. nitro or sulphonyl), the groups G and M being electronically linked through the conjugated array of multiple bonds. One example of such as chromophore is the dansyl (1-dimethylamino-5-naphthylsulphonyl) group.

The precise nature of the chromophore, its chemical properties and its absorbance characteristics, can be determined on a case by case basis depending upon the type of chemistry to be used in a given synthesis. It is most preferred however, that the chromophore is inert to the reactants and reaction conditions employed in the synthesis.

Presently preferred chromophores include anthracenyl and dansyl (5-dimethylamino-1-naphthylsulphonyl groups, anthracenyl being particularly preferred.

The quantitation of the substrate R can be either absolute quantitation or relative quantitation in which the relative amounts of the substrate R and another component in the synthesis mixture (e.g. another substrate, or starting material, or a side product for example) are determined. Relative quantitation makes use of the fact that the chromophore C^u confers on each substrate a common set of characteristic absorbances that can be used as the basis for the spectrophotometric analysis.

In one embodiment of the invention, the analysis is carried out on a single bead, or a small number of beads, for example from 1 to 20 beads, more usually less than ten beads. Such beads typically have an average diameter in the range from 90μm to 250μm and a compound loading of between 0.1 mmol g^{-1} and 0.4 mmol g^{-1}average loading of an individual bead is less than 10 nanomoles, for example less than 5 nanomoles, e.g. approximately 1 nanomole.

Accordingly, in one preferred embodiment, the invention provides a method of analysis of a solid phase construct; which method comprises:

(i) providing a chemical construct comprising a solid support Q (e.g. a resin bead having an average diameter in the range from 90μm to 250μm) having linked thereto via a connecting group Y a substrate R, the substrate R being present on each solid support in an amount of no more than 10 nanomoles, for example less than 5 nanomoles, e.g. less than 2 nanomoles; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectrophotometry;

(ii) isolating a solid support, or a plurality of solid supports not exceeding 20 in number (for example less than 10, more particularly less than 5, e.g. 1 solid support);

(ii) treating the solid support(s) to cleave the connecting group at the first cleavage site to release the fragment Fr^u containing the substrate R; and

(iii) subjecting the fragment Fr^u to ultra violet, visible or fluorescence spectrophotometric analysis to quantify the substrate R.

A significant advantage of the use of the UV chromophore-containing constructs of the present invention is that they can be used to provide quantitative information on very small amounts of substrate compounds, for example amounts of the order of 1 nanomolar typically available from single resin beads of the size used in split and mix combinatorial methods. In combinatorial libraries formed by the split and mix method, each bead typically will contain only a single substrate compound, but a library will contain a plurality of beads bearing different substrates. The analysis of mixtures of beads bearing different substrates is problematical and hence it is generally necessary to carry out analyses on single beads. The constructs of the present invention enable analysis to carried out efficiently and accurately at the single bead level, and in particular with beads of a size of the order of 90μm to 250μm in diameter with loadings of the order of 0.1- 0.4 mmol g^{-1}.

The incorporation of a chromophore into the connecting group Y enables the substrate to be subjected to quantitative analysis. However, it is preferred that the fragment Fr^u also contains a mass spectroscopic sensitising group G as well as the chromophore C^u in order to facilitate qualitative analysis of the substrate as well as quantitative analysis.

The sensitising group G renders the fragment Fr, and hence indirectly the substrate R, more sensitive to analysis by mass spectrometric techniques. Thus the sensitising group can be a group which is readily ionisable under the conditions encountered in a mass spectrometer, and in particular electrospray mass spectrometry, to afford a strong signal. The inclusion of ionisable sensitising groups serves to ensure that the fragment is ionised sufficiently in the mass spectrometer to give a strong response. This overcomes a problem inherent in many molecules synthesised by solid phase methods where a suitable ionising group is not present and analysis by high throughput mass spectral techniques is problematical.

The ionisable group can be for example a basic amino group or a carboxylate group but preferably it is a basic amino group. It will be appreciated that the term "basic amino group" as used herein refers in particular to an amino group, which is readily protonated.

The basic amino group can be a primary amino group, a secondary amino group, or a tertiary amino group. Where the basic amino group is a tertiary amino group, it can be for example, a cyclic amino group such as piperidino, piperazino, pyrrolidino, or morpholino, piperidino or piperazino (e.g.N-methylpiperazino) being presently preferred.

In one preferred embodiment of the invention, the chemical construct is configured such that cleavage at the first cleavage site forms or introduces on the chemical fragment Fr^u at the first cleavage site a moiety comprising the mass spectral or other sensitising group G. It will be recognised that In such constructs, the sensitising group G is formed or introduced by cleavage of the "skeleton" of the construct, and not by cleavage of a side chain or removal of a protecting group from a pendant sensitising group. An advantage of forming the sensitising group G in this way is that it avoids the potential problem of a pre-existing or pre-formed sensitising group interfering with the chemistry of the construct. Moreover, it avoids the need to have the sensitising group independently protected thereby obviating the additional problem of de-protection, a requirement which could limit the types of chemistry available to the chemist for cleavage at the first and second cleavage sites. Thus, for example, if a protected pendant sensitising group were present, and for example the conditions required for de-protection involved the use of an acid such as trifluoroacetic acid, then this would effectively prevent the use of an acid labile cleavage site as the second cleavage site linking the substrate to the rest of the construct. The constructs of the invention avoid such problems.

The atoms or functional group making up the sensitising group G can be present in a masked form in the construct before cleavage of the fragment Fr^u from the resin, the cleavage conditions merely serving to unmask the sensitising group G. Alternatively, the atoms or functional group making up or containing the sensitising group G can be introduced at the cleavage site during the cleavage reaction. For example, where the sensitising group is a basic amino group, the nitrogen atom of the amino group can be present in the construct before cleavage, or it can be introduced during the cleavage reaction.

When the sensitising group G is introduced from an external source during the cleavage reaction, it can form part of a larger chemical moiety. For example, the group G can be introduced as part of a group X-G wherein X is the residue of a nucleophile or electrophile, for example a nitrogen or sulphur-based nucleophilic group, e.g. a group of the formula G-Alk-Nuc wherein Alk is an alkylene group (e.g. a C_{2-20} and preferably C_{2-6} alkylene group) optionally interrupted by one or more heteroatoms selected from oxygen, nitrogen and sulphur, and Nuc is a nucleophile, such as an amine (e.g. NH or NR' where R' is a C_{1-6} alkyl group) or a thiolate group.

It is preferred that the chemical fragment Fr^u contains a means for imparting a characteristic signature to the mass spectrum of the fragment. The signature can advantageously be provided by incorporating into the fragment a "peak splitting" isotopic label. The peak splitting isotopic label comprises at least one atom that exists in a number of stable isotopic forms. By isotopic labelling of one or more particular atoms in the fragment Fr^u such that a given atom is labelled with a mixture of isotopes, the mass spectra of the molecular ions will appear as a characteristic pattern, the precise pattern depending on the relative amounts of the individual isotopes. Thus, for example, if a given atom in the fragment Fr is labelled such that 50% of the atoms are of one isotopic form and 50% are of another isotopic form, the mass spectrum will show the molecular ion as a characteristic doublet in which the peaks are of approximately equal height.

The purpose of the peak splitting atom(s) is to provide a characteristic pattern, which will characterise any peak in the mass spectrum originating from the analytical fragment Fr, thereby distinguishing such peaks from those due to extraneous materials.

Examples of atoms that can be used as isotopic peak splitting labels include $^{1}H/^{2}H$ (D), $^{79}Br/^{81}Br$, $^{12}C/^{13}C$, $^{14}N/^{15}N$ and $^{16}O/^{18}O$.

The fragment Fr^u can contain a single peak splitting isotopic label or more than one such label. For example, the isotopic label can be a single bromine atom in which case the peak for the molecular ion of the analytical fragment Fr^u liberated following cleavage from the solid support will appear as a doublet. By introducing a second or subsequent peak splitting label(s), a more complex peak pattern will be produced for the molecular ion.

The isotopic peak splitting label(s) preferably is/are located between the first and second cleavage sites.

The first and second cleavage sites can be defined by first and second linker groups L^1 and L^2. A "spacer group" A can be interposed between the two linker groups L^1 and L^2, the spacer group A containing or having linked thereto the UV chromophore C^u. The group A typically also contains an isotopic peak splitting label. Accordingly, in one preferred embodiment of the invention, there is provided a chemical construct as hereinbefore defined wherein the connecting group Y has the formula L^1-A-L^2; wherein L^1 is a first linker group defining the first cleavage site; A is a chemical group (the spacer group) containing or having linked thereto the UV chromophore C^u, and optionally containing a peak splitting isotopic label; and L^2 is a second linker group defining the second cleavage site.

The first and second cleavage sites are orthogonally and selectively cleavable; i.e. the conditions used to effect cleavage at one of the cleavage sites will not cleave the other. A wide variety of different types of cleavage reaction can be used, examples being reactions selected from acid catalysed cleavage, base catalysed cleavage, oxidative cleavage, reductive cleavage, nucleophilic displacement, electrophilic displacement, and thermal, photochemical and enzymatic cleavage.

Thus, in a preferred embodiment, there is provided a chemical construct as hereinbefore defined wherein the first cleavage site is selectively cleavable by one type of chemistry selected from a group of chemistries consisting of cleavage under acid conditions, base catalysed cleavage, oxidative cleavage, reductive cleavage, nucleophilic displacement, electrophilic displacement, and thermal or enzymatic cleavage, and the second cleavage site is selectively cleavable by a different type of chemistry selected from the said group.

Either the first or second cleavage sites/linkers, or both, can be of the "safety catch" variety; i.e. the cleavage site or linker group must be chemically modified in a first step before it can be subjected to cleavage in a second step. An advantage of such an arrangement is that it prevents or significantly reduces the possibility of cleavage taking place inadvertently. One example of a "safety catch" mechanism involves oxidation of a functional group in a first step, the oxidation serving to make the functional group more amenable to displacement by a nucleophile in a subsequent cleavage step. The nucleophile can vary considerably in structure. For example, in one embodiment, the nucleophile can be an amino group-containing nucleophile), the amino group participating in the nucleophilic displacement action such that the amino group is attached directly to the cleavage site. Alternatively, in another embodiment, the amino group (or other sensitising group) can be present in a group (e.g. a dialkylaminoalkyl-thiolate anion) containing another nucleophile such as a sulphur nucleophile, which becomes attached to the cleavage site.

Examples of linkers that can be selectively cleaved under acidic conditions include appropriately substituted benzyloxycarbonyl groups and appropriately substituted diphenyl-methylamino groups, both of which can be cleaved by the action of trifluoroacetic acid.

Particular examples of acid cleavable linker groups are set forth in *The Combinatorial Index*, Barry A. Bunin, Academic Press, San Diego 1998, the disclosure of which is incorporated herein by reference. Linkers of the "Rink" or "Knorr" type typically comprise an N-protected 1-amino-1,1-diphenylmethane moiety, the amino group when deprotected allowing attachment to a substrate, one of the phenyl rings being substituted for example with dimethoxy groups and the other having a carboxyalkyloxy substituent providing a second point of attachment. Cleavage with TFA gives rise to a substrate compound having a terminal carboxamido group. Linkers of the "Wang" type typically contain a substituted phenoxyacetyl group, the acetyl group providing one point of attachment, and a benzylic hydroxyl group on the phenyl ring forming a second point of attachment. Esters can be formed between a carboxyl group of a substrate and the benzylic hydroxyl group, the ester groups being subsequently cleavable with TFA to release a substrate compound having a terminal carboxylate group.

Examples of linker groups that can be cleaved by nucleophilic displacement include sulphonamide linker groups in which the aryl (e.g. phenyl) ring of the sulphonamide contains an electron withdrawing group such as a nitro group, preferably in the *ortho* position of the aryl

ring. Cleavage of the linker between the amino and sulphonyl moieties can be effected by a nucleophile such as a thiol or thiolate nucleophile in the presence of a base such as 1,8-diazobicyco[5.4.0]undec-7-ene (DBU), morpholine or sodium carbonate. Further examples of groups that can be cleaved by nucleophilic displacement include mercaptopyrimidine-based "safety catch" linkers such as 5-carboxy-2-mercaptopyrimidine, where cleavage can be effected by reacting under oxidising conditions to generate a sulphoxide or sulphone linkage, followed by reaction with a nucleophilic amino group to form a 2-aminopyrimidine. Such linkers can be cleaved, for example, by reaction with a cyclic amine such as piperidine or an N-substituted piperazine (e.g.N-methylpiperazine), or an amino group-containing thiolate nucleophile (e.g. dimethylaminoethylthiolate) after first oxidising with an oxidising agent, preferably a realtively mild oxidising agent such as a per-acid or an inorganic per-salt such as potassium peroxymonosulphate (e.g. Oxone™).

Examples of linkers that can be selectively cleaved under basic conditions include groups containing ester linkages.

In one embodiment of the invention, the first cleavage site (which can be , for example, defined by a sulphonamide linker) is selectively cleavable by nucleophilic displacement and the second cleavage site (which can be, for example, defined by a Rink linker) is selectively cleavable under acidic conditions.

In another embodiment of the invention, the first cleavage site (which can be defined by, for example, an ester linkage) is selectively cleavable by base and the second cleavage site (which can be, for example, defined by a Rink linker) is selectively cleaved under acidic conditions.

By making the first and second cleavage sites, e.g. as defined by the first and second linker groups L^1, L^2, orthogonally cleavable, it is possible selectively to separate from the construct either the chemical fragment Fr^u or the substrate R, simply by using different cleavage conditions. This means that during experiments designed to optimise the conditions for a particular reaction step, the chemist can subject the construct to conditions suitable for cleaving off the analytical fragment Fr^u thereby allowing analysis to be carried out to determine the outcome of each test reaction. Similarly, during a preparative reaction (e.g. preparation of a combinatorial library or subsequent preparative reactions such as scale-up reaction or commercial production), quality control (QC) can be carried out by removing a

number of individual solid supports from the reaction vessel, cleaving the constructs at the first cleavage site and analysing the resulting fragment Fr^u to see whether a particular reaction step has worked. On the other hand, by cleaving at the second cleavage site e.g. on the second linker group, rather than the first, the substrate R is released from the solid support. Thus, an advantage of the constructs of the present invention is that they can be used both at an experimental level to optimise a particular process step or for QC purposes, and also at a preparative level to release exclusively the substrate for screening purposes, without modifying the linker groups.

In one embodiment of the invention, the fragment Fr^u, and more preferably the spacer group A contains an alkylene diamine group or aminoalkoxy group. The precise size of the alkylene group and its degree of substitution is not currently considered to be important, but by way of example the chain could be from 2 to 30 carbon atoms in length, for example up to 20 carbon atoms, more typically less than 10 carbon atoms, particular examples being ethylene or propylene diamine or amino alcohol groups which may be substituted or unsubstituted. The alkylene diamine group or amino alcohol typically contains a peak splitting isotopic label as hereinbefore defined. The two amino groups can each be bonded to respectively the first and second linker groups. In order to increase the mass of the spacer group A, the alkylene diamine group can be substituted by an aryl group, for example an N-aryl group such as an N-benzyl group. The N-aryl group may optionally be substituted with one or more substituent groups.

The chromophore C^u may be formed as an integral part of the backbone or skeleton of the fragment Fr^u (e.g. as part of the spacer group A), or it can be a pendant group. Thus, for example, when it is a pendant group, it can be linked to A either directly or via an alkylene chain (e.g. of 1 to 6 carbon atoms) optionally interrupted by one or more oxygen, nitrogen or sulphur atoms, or by means of an ether, thioether, amide, sulphonamide or ester linkage. Where the spacer group A contains an alkylene diamine group or aminoalkoxy group, the chromophore C^u can, for example, be linked (directly or via an optionally interrupted alkylene chain as hereinbefore defined) to the nitrogen atom of the, or one the, amino groups. For example, the chromophore C^u can be linked to the nitrogen atom by means of an ethylene or propylene chain or by a sulphonyl group.

Where, as is preferred, the spacer group contains one or more mass-spectral peak splitting isotopic labels, these can be located either in the alkylene chain or in a substituent

group attached to the alkylene chain. Thus, for example, an N-benzyl group bonded to one of the two amino groups in an alkylenediamine can have a methylene group, which is substituted with the peak splitting atom deuterium. Alternatively, an aryl ring (e.g. an N-benzyl group) present in a substituent on the alkylene diamine can be substituted with a peak splitting bromine atom, one particular example of an aryl group being an N-*o*-bromobenzyl group. In compounds where a substituent such as a benzyl group is attached to one of the amino groups of an alkylene diamine spacer group A, the chromophore C^u can be linked, directly or indirectly, to the other of the amino groups. In one currently preferred embodiment, the chromophore C^u is a polycyclic aryl group such as an anthracenyl or phenanthrenyl group linked to a nitrogen atom at one end of the diamine spacer group.

Although alkylene diamine and amino alcohol groups are given as specific examples of spacer groups, alternative spacer groups can be used. For example, the spacer groups can be formed from hydrocarbon chains containing up to thirty or more carbon atoms in the chain which can be optionally interrupted with one or more heteroatoms such as oxygen, nitrogen or sulphur. As a further alternative, the spacer can be for example a peptide chain containing one or more amino acids. The precise nature and length of the spacer is not currently considered to be important provided that the spacer does not interfere with the chemistry of the construct.

The solid support Q can be any type of solid support suitable for use in solid phase synthesis, and in particular combinatorial chemistry. Thus, purely by way of example, the solid support can take the form of beads, a solid surface, solid substrates, particles, pellets, discs, capillaries, hollow fibres, needles, solid fibres, or organic or inorganic gels such as silica gels, and insoluble organic particles such as particles formed from fullerenes.

Examples of beads are polymeric beads such as cellulose beads or resin beads, particular examples of materials from which resin beads can be prepared including functionalised polymer resins such as polystyrene resins, polyacrylamide resins and dimethylacrylamide resins. Examples of suitable supports are listed in *The Combinatorial Index* by Barry A. Bunin, referred to above.

As set out hereinabove, the invention also provides a method of analysing the constructs hereinbefore defined; the method comprising cleaving the construct at the first cleavage site to release the chemical fragment Fr^u and subjecting the fragment to UV, visible or fluorescence spectrometric analysis, for example quantitative UV analysis.

The cleavage products can be subjected to chromatography, preferably a liquid chromatography technique such as HPLC, using a UV detector which can be set up to provide a means of determining concentrations of the various compounds as they elute from the chromatography column. The chromatography column can in turn be linked to another instrumental means of analysis for the purpose of identifying the substrate. A preferred means of instrumental analysis is mass spectroscopic analysis, and thus the method and constructs of the invention afford a means of means of achieving both identification and quantitation of the substrate in a single linked series of analytical procedures

Preferably, the mass spectrometric analysis is a "soft" mass spectrometric technique such as MALDI or electrospray or ion cyclotron mass spectrometry. In this embodiment, the construct typically contains a mass spectrometric sensitising group as hereinbefore defined.

In a particularly preferred embodiment, the construct is configured such that cleavage at the first cleavage site generates on the chemical fragment Fr^u a mass spectrometric sensitising group (e.g. a group which is ionisable under mass spectroscopic conditions), and then subjecting the chemical fragment to mass spectrometry, e.g. electrospray mass spectrometry.

The analysis of the fragment Fr^u provides information on the reaction history of the construct. Thus, by mass spectrometric analysis, it can readily be determined whether or not the desired substrate has been formed in a given reaction sequence. Analysis of the fragment Fr^u can therefore be used not only to characterise the substrate or product of the solid phase reaction sequence, but also to follow the progress of the reactions. Quantitation of the reaction products is achieved by means of the UV chromophore.

In a further aspect, the invention provides an intermediate chemical construct for use preparing a chemical construct as hereinbefore defined, the intermediate construct having the formula Q-Y' wherein Y' is a reactive or protected form of the group Y, and Q and Y are as hereinbefore defined.

In a still further aspect, the invention provides an intermediate construct of the formula Q-L^1-AP wherein Q and L^1 are as hereinbefore defined and AP is a reactive or protected form of the spacer group A containing a chromophore C^u and optionally a splitting isotopic label.

In a particular embodiment, the intermediate construct has the general formula Q-L^1-N(Alk-C^u)-Alk-NH-X^1 wherein Alk is an alkylene group and X^1 is hydrogen or an aralkyl group. The intermediate construct is preferably isotopically labelled with a peak splitting combination of atoms such as $^1H/H^2$ (D), $^{79}Br/^{81}Br$, $^{12}C/^{13}C$, $^{14}N/^{15}N$ and $^{16}O/^{18}O$.

The invention further provides a method of identifying a pharmaceutically useful substrate comprising preparing a library containing a plurality of chemical constructs as hereinbefore defined and subjecting the library to biological testing to identify biologically active substrates. The method may include the further step of formulating a biologically active substrate thus identified with a pharmaceutically acceptable carrier to form a pharmaceutical composition.

Description of Illustrative Embodiments

The invention will now be illustrated but not limited by reference to the following examples.

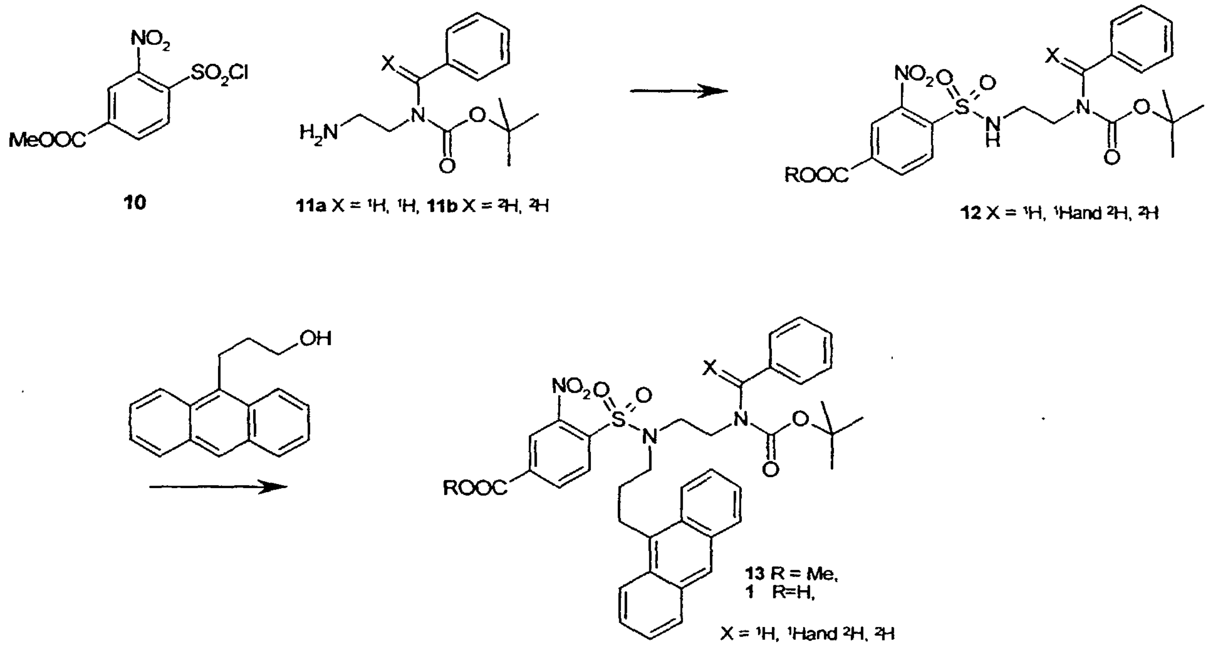
EXAMPLE 1

Preparation of a Construct Containing an Anthracenyl UV Chromophore

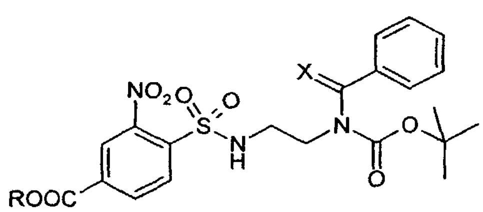
In order to demonstrate that UV spectrometry can be used to provide accurate quantitative information about the products of solid phase reactions, a construct containing an anthracenyl UV chromophore was prepared according to the synthetic route shown in Schemes 1, 2 and 3 below. The construct was provided with a sulphonamide linker at the first cleavage site and a Rink linker at the second cleavage site, an ethylene diamine chain connecting the two linkers and the two nitrogen atoms of the diamine chain serving as the points of attachment of the UV chromophore and either a peak splitting deuterium labelled benzyl group or an unlabelled benzyl group.

Scheme 1 below illustrates the synthesis of a non-resin bound intermediate construct, Scheme 2 illustrates the attachment of the construct of Scheme 1 to a resin support and subsequent reactions on the resin, and Scheme 3 illustrates the attachment of different substrate groups to the constructs and subsequent cleavage experiments. In the experimental section below, the compound numbers used relate to the compounds identified in Schemes 1, 2 and 3.

Scheme 1



Experimental for Scheme 1



12 X = ¹H, ¹Hand ²H, ²H

Preparation of Compound 12 (X = ^{1}H, ^{1}H)

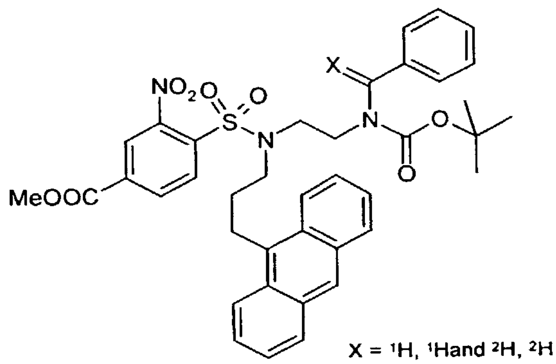
Dry triethylamine (25 µl, 0.2 mmol) was added to a solution of the amine (X=H, H) **(11)** (0.05 mmol) in dry dichloromethane (1 ml). A solution of 2-nitro-4-methylbenzoatesulfonyl

chloride (**10**) (25 mg, 0.1 mmol) in dry dichloromethane (0.5 ml) was then added dropwise and the mixture stirred for 15 minutes. The solvent was evaporated *in vacuo* and the residue purified by flash chromatography using 2:1 hexane/ethyl acetate as eluent to afford the sulphonamide (**12**) (23.3 mg, 95%) as a colourless oil, R_f 17/50 [hexane-ethyl acetate (2:1)]; (Found: C, 53.7; H, 5.4; N, 8.4; S, 6.5. $C_{22}H_{27}N_3O_8S$ requires C, 53.5; H, 5.5; N, 8.5; S, 6.5%); δ_H (DMSO-d^6) 1.38 (9H, s, H-18), 3.10 (2H, t, *J* 8, H-9), 3.24 (2H, t, *J* 8, H-10), 3.95 (3H, s, O-Me), 4.32 (2H, s, H11), 7.13-7.32 (5H, m, H-13, 14 and 15), 8.09 (1H, d, *J* 8, H-6), 8.29 (1H, s, H-3), 8.32 (1H, d, *J* 8, H-3); δ_C (DMSO-d^6) 28.3 (C-18), 40.2 (C-9 and 10), 46.2 (C-11), 53.2 (O-Me), 79.3 (C-17), 125.1 (C-13), 127.2 (C-14 and 15), 128.6 (C-6), 130.3 (C-13), 133.1 (C-5), 134.4 (C-12), 136.6 (C-1), 147.6 (C-4), 163.7 (C-16 and C=O); MS (Electrospray -ve) m/z 494 $(M-H)^-$; HPLC, R_t 6.8 min.

Preparation of Compound 12 (X = ^{2}H, ^{2}H)

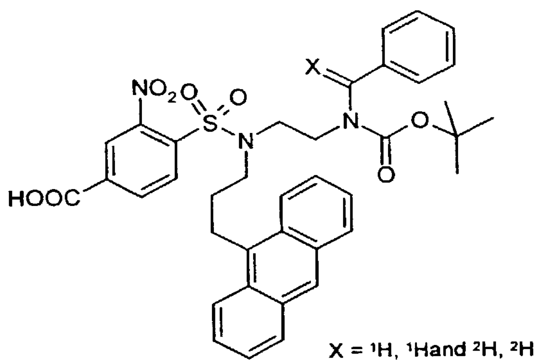
Dry triethylamine (25 µl, 0.2 mmol) was added to a solution of the amine (X=^{2}H, ^{2}H) (**11**) (0.05 mmol) in dry dichloromethane (1 ml). A solution of 2-nitro-4-methylbenzoatesulfonyl chloride (**10**) (25 mg, 0.1 mmol) in dry dichloromethane (0.5 ml) was then added dropwise and the mixture stirred for 15 minutes. The solvent was evaporated *in vacuo* and the residue purified by flash chromatography using 2:1 hexane/ethyl acetate as eluent to afford the sulphonamide (**12**) (23.5 mg, 94%) as a colourless oil, R_f 17/50 [hexane-ethyl acetate (2:1)]; (Found: C, 53.2; H, 5.6; N, 8.5; S, 6.4. $C_{22}H_{25}D_2N_3O_8S$ requires C, 53.3; H, 5.5; N, 8.5; S, 6.5%); δ_H (DMSO-d^6) 1.40 (9H, s, H-18), 3.05 (2H, m, H-9), 3.20 (2H, dt, *J* 3 and 13, H-10), 3.9 (3H, s, O-Me), 7.10-7.35 (5H, m, H-13, 14 and 15), 8.09 (1H, d, *J* 8, H-6), 8.35 (1H, d, *J* 8, H-5), 8.40 (1H, m, H-8), 8.45 (1H, s, H-3),; δ_C (DMSO-d^6) 27.8 (C-18), 40.9 (C-9 and 10), 45.9 (C-11), 53.0 (O-Me), 79.1 (C-17), 124.8 (C-13), 127.0 (C-14 and 15), 128.3 (C-6), 130.0 (C-13), 132.9 (C-5), 134.2 (C-12), 136.4 (C-1), 147.5 (C-4), 154.7 (C-2), 163.5 (C-16 and C=O); MS (Electrospray +ve) m/z 496 $(MH)^+$; HPLC, R_t 6.8 min.

Preparation of Compounds 13 and 1



Compound 13

Di-*tert*-butylazodicarboxylate (32.6 mg, 142 μmol) was dissolved in dry tetrahydrofuran (0.5 ml) under nitrogen and added dropwise to a stirred solution of triphenylphosphine (37.1 mg, 142 μmol), 3-(9-anthracene)propanol (17.6 mg, 74 μmol) and sulphonamide **12** (X=H, H and ^{2}H, ^{2}H) (35 mg, 71 μmol), dissolved in dry tetrahydrofuran (1 ml) under nitrogen. After one hour the reaction was deemed complete by tlc and the solution was evaporated to near-dryness. The residue was purified by flash chromatography

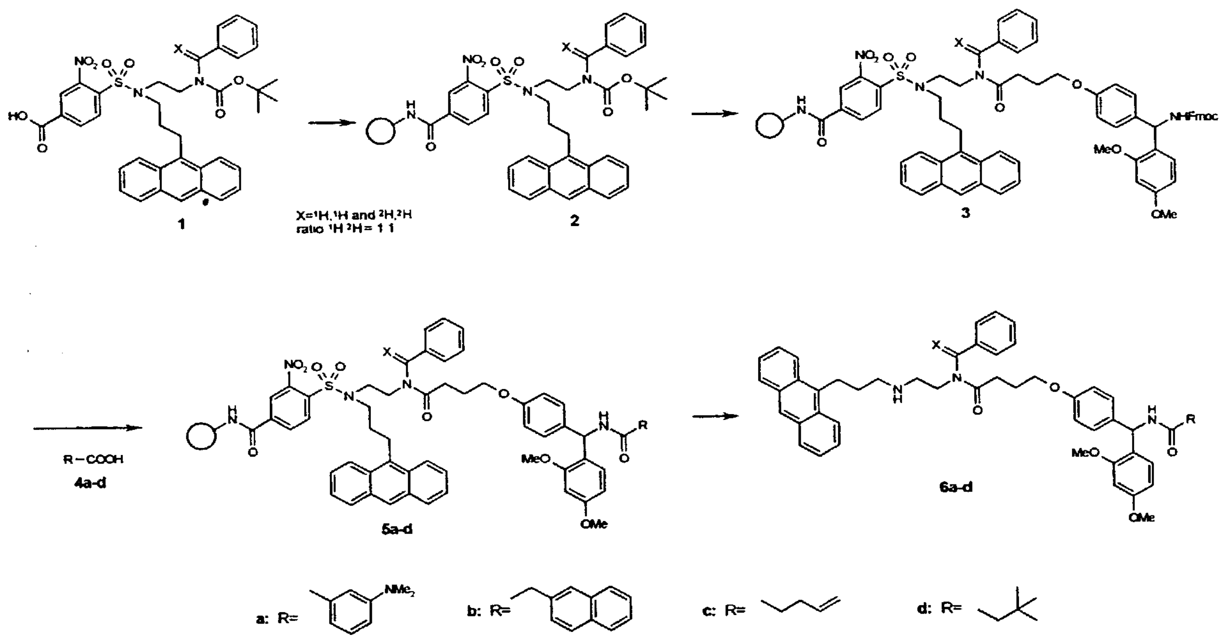


Compound 1

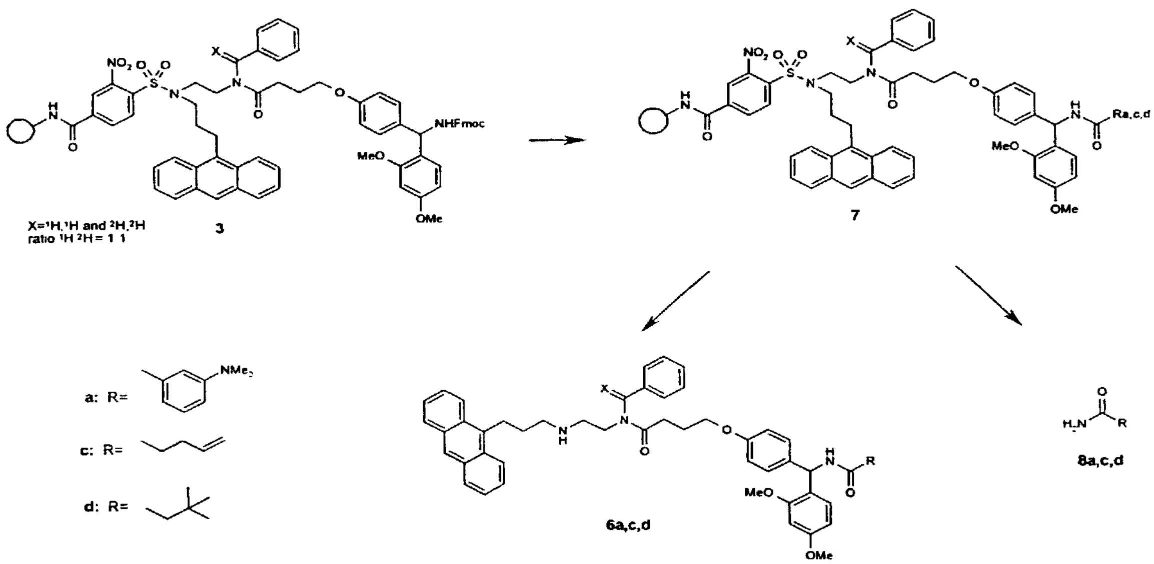
using 2:1 hexane/ethyl acetate containing 10% triethylamine to afford the sulphonamide **13** (45 mg, 90%) as a colourless oil, R_f 15/38 (hexane-ethyl acetate (2:1)); MS (Electrospray +ve) m/z 612, 614 $(MH\text{-}Boc)^+$, MS (Electrospray –ve) m/z 756, 758 (M-H+formate) HPLC, R_t 6.9 min.

2M aqueous sodium hydroxide (0.35 ml, 0.7 mmol) was added dropwise to a solution of the ester **13** (0.25 g, 0.35 mmol) in methanol-1,4-dioxane (4:1) (2 ml). After stirring at room temperature for 1 hour (further methanol-dioxane (ca. 1 ml) was added as necessary to ensure a single phase) sufficient 2M HCl added to render the solution mildly acidic. The solution transferred to a separating flask containing ethyl acetate (50 ml) and water (10 ml) and after partition the aqueous layer was extracted again with ethyl acetate (25 ml). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure to afford the acid **1** (0.23 g, 94%) as a colourless oil; R_F 2/40 [hexane-ethyl acetate (2:1)]; δ_H ($CDCl_3$) 1.40 (9H, br. d, *t*Bu), 1.90 (2H, br. m, CH_2), 3.3-3.7 (8H, br. m, 4xCH_2), 7.10-8.30 (17H, br. m, Arom. H); MS (electrospray +ve) m/z 697, 699 HPLC, R_t 7.2 min.

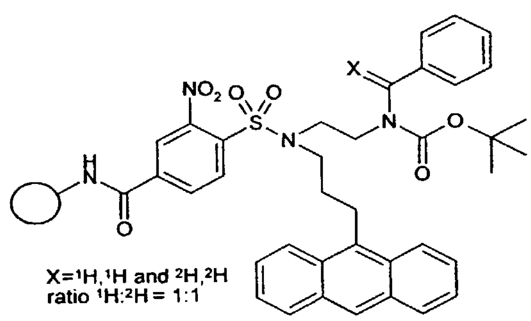
Scheme 2



Scheme 3



Experimental for Schemes 2 and 3



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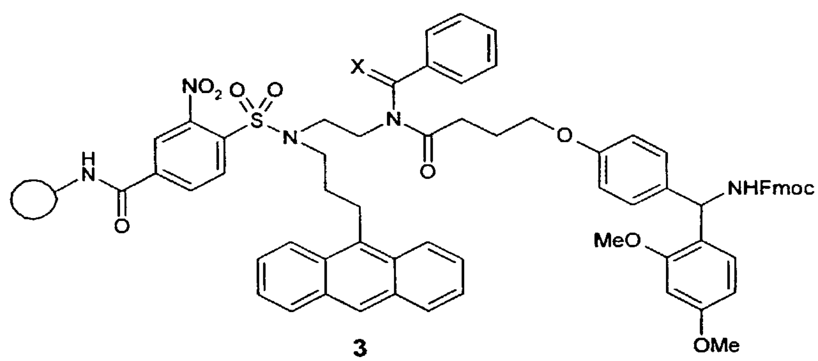
Dry aminomethyl Argogel® (0.87 g, 0.35 mmol)), having an average bead diameter of 150 μm and a loading of 0.4 mmol g^{-1}, in a reaction vessel flushed with dry nitrogen was swollen with dry dichloromethane (5 ml). In a separate flask flushed with nitrogen, the benzoic acid (**1**) (0.67 g, 0.93 mmol), PyBOP (0.728 g, 1.4 mmol) and 1-hydroxy-benzotriazole (0.19 g,

1.4 mmol) were dissolved in dry dimethylformamide (2 ml)and the resulting solution stirred two minutes before being transferred to the suspension of resin *via cannula.* A slow stream of nitrogen was aspirated through the solution for five minutes to ensure mixing and diisopropylethylamine (0.49 ml, 2.8 mmol) then added. Agitation was continued for 18 h. whereupon the resin (**2**) was drained, washed (dichloromethane (3x7 ml), dimethylformamide (3x7 ml) and dichloromethane (3x7 ml)) and dried *in vacuo*. An analytical sample of **2** gave a negative Kaiser test.

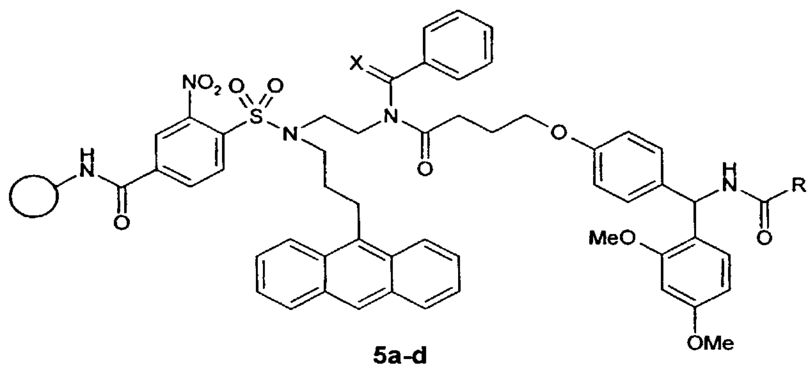


3

Resin **2** (0.35 mmol) was swollen in dichloromethane (3 ml) and phenol (66 mg, 0.7 mmol) added, followed by trifluoroacetic acid (4.5 ml). The resin was gently agitated for 5 minutes using a stream of nitrogen then drained and washed with dichloromethane (2x5 ml). The process was repeated and, after draining, the resin washed (dichloromethane (3x8 ml), 10% diïsopropylamine in dichloromethane (2x6 ml) and dichloromethane (4x8 ml)) and dried *in vacuo*. An analytical sample gave a positive Kaiser test.

The resin, in a reaction vessel flushed with nitrogen, was swollen with dry dichloromethane (5 ml). In a separate flask flushed with nitrogen C-4 RINK acid (0.795 g, 1.4 mmol), PyBOP (1.46 g, 2.8 mmol) and 1-hydroxybenzotriazole (0.378 g, 2.8 mmol) were dissolved in dry dimethylformamide (3 ml) and the resulting solution transferred to the suspension of resin *via cannula.* This mixture was agitated gently by aspirating a stream of nitrogen through the solution and after five minutes dry diisopropylethylamine (0.98 ml, 5.6 mmol) was added. Agitation was continued on a mechanical shaker for 18 h. whereupon the resin (**3**) was drained and washed (dichloromethane (3x8 ml), dimethylformamide (3x5 ml) and dichloromethane (3x5 ml)) and dried *in vacuo*. An analytical sample gave a negative Kaiser

test.



5a-d

A sample of resin **3** (50 mg, 14 μmol) was treated with 20% v/v piperidine in 1:1 dichloromethane/dimethylformamide (2 ml) for 15 minutes. The resin was drained and washed (dichloromethane (3x2 ml), dimethylformamide (3x2 ml) and dichloromethane (3x2 ml)) then dried *in vacuo*, an analytical sample giving a positive bromophenol blue test. The reaction vessel containing the resin was flushed with nitrogen and dry 1:1 dichloromethane /dimethylformamide (0.5 ml) added. In a separate flask flushed with nitrogen PyBOP (57 mg, 110 μmol), 1-hydroxybenzotriazole (15 mg, 110 μmol) and the appropriate acid **(4a-d)** (55 μmol) were dissolved in dry 1:1 dichloromethane-dimethylformamide (0.5 ml) and this solution added in one portion to the suspension of resin, through which a stream of nitrogen was aspirated to ensure uniform mixing. After agitating for 18 h. the resin was drained, washed (dichloromethane (3x2 ml), dimethylformamide (4x2 ml) and dichloromethane (3x2 ml)) and dried *in vacuo* to give resins **5a-d**. An analytical sample of each gave a negative Kaiser test.

A small sample of each of the four resins was treated with DBU/ mercaptoethanol in acetonitrile to release **6a-d** which were analysed by HPLC and low-resolution mass spectrometry:

6a (R = 3-dimethylaminobenzoyl) HPLC R_t 4.51 min., MS (electrospray +ve) *m/z* 843.7 and 845.7;

6b (R = 2-naphthylacetoyl) HPLC R_t 5.41 min., MS (electrospray +ve) *m/z* 863.4 and 865.4;

6c (R = 4-pentenoyl) HPLC R_t 4.98 min., MS (electrospray +ve) *m/z* 778.7 and 780.7;

6d (R = *tert*-Butylacetoyl) HPLC R_t 5.18 min., MS (electrospray +ve) *m/z* 795.0 and 797.0.

Relative quantitation.

An experiment was carried out to establish whether UV analysis could be used to quantify the relative concentrations of target compounds or substrates in a multicomponent mixture.

Approximately equal quantities (ca. 50 mg) of resins **5a** and **5b** were combined and treated with 4-mercaptobenzoic acid (43 mg, 20 μmol) and morpholine (49 μmol) in dichloromethane (2 ml). After one hour the solution was drained and the resin washed with dichloromethane (2x1 ml). These combined washings were diluted with ethyl acetate (10 ml) and washed firstly with 50% saturated aqueous sodium bicarbonate (3 ml), water (3 ml) and brine (2 ml) and dried over magnesium sulphate. The filtered solution was evaporated to afford approximately 5 mg of a mixture of **6a** and **6b**. The ratio of components in this mixture was assessed firstly by HPLC, measuring peak areas at 254 nm and 386 nm, which was correlated with the ratio determined from the magnitude of the integrations of characteristic resonances in the ^{1}H NMR spectrum.

HPLC analysis

	6a	6b
Area ratio (254 nm)	1.00	1.30
Area ratio (386 nm)	1.00	1.26

^{1}H nmr analysis

	6a	6b
Ratio	1.00	1.33

In the same way a mixture of resins **5c** and **5d**, afforded a mixture of **6c** and **6d**, the ratio of which were again assessed by HPLC and correlated with the ratios measured using ^{1}H nmr spectroscopy.

HPLC analysis

	6c	6d
Area ratio (254 nm)	**1.00**	**0.93**
Area ratio (386 nm)	**1.00**	**0.97**

^{1}H nmr analysis

	6c	6d
Ratio	**1.00**	**0.96**

Finally, **6a**, **6b**, **6c** and **6d** were combined in one solution, which was analysed using HPLC and ^{1}H nmr as before:

HPLC analysis

	6a	6b	6c	6d
Area ratio (254 nm)	**0.87**	**1.35**	**1.00**	**1.00**
Area ratio (386 nm)	**0.84**	**1.30**	**1.00**	**0.99**

^{1}H nmr analysis

	6a	6b	6c	6d
Ratio	**0.85**	**1.34**	**1.00**	**1.05**

As can be seen from the data provided, in each case the ratios obtained from the UV analysis correlated closely with the ratios obtained from nmr analysis, thereby demonstrating the accuracy of the UV method as a means of quantifying the products of the solid phase synthesis.

In the analytical experiments described above , the mixtures of products analysed were prepared simply by mixing together different resins, each containing a single substrate. In order to demonstrate that the UV analysis method is also applicable to the analysis of reaction mixtures in which multiple reaction products or substrates are formed, a further series of experiments was carried out.

Resin **3** (214 mg, 59 μmol) was treated with 20% v/v piperidine in 1:1 dichloromethane/dimethylformamide (3 ml) for 60 minutes. The resin was drained, washed (dichloromethane (3x3 ml), dimethylformamide (3x3 ml) and dichloromethane 93x3 ml)) and

dried *in vacuo*, an analytical sample giving a positive bromophenol blue test. The reaction vessel was then flushed with nitrogen and the resin swollen with dry 1:1 dichloromethane-dimethylformamide (2 ml).

In a separate flask flushed with nitrogen PyBOP (245 mg, 470 μmol), 1-hydroxy-benzotriazole (64 mg, 470 μmol) and a mixture of 4-pentenoic acid (6.0 μl, 59 μmol), 3-dimethylaminobenzoic acid (19.5 mg, 120 μmol) and *tert*-butylacetic acid (23 μl, 180 μmol) were dissolved in dry 1:1 dichloromethane/dimethylformamide (1 ml) and the solution added in one portion to the suspension of resin. A gentle stream of nitrogen was passed through the mixture and after five minutes diisopropylethylamine (165 μl, 940 μmol) was added. After agitating for 18 h. the resin (7) was drained, washed (dichloromethane (3x4 ml), dimethylformamide (4x4 ml) and dichloromethane (3x4 ml)) and dried *in vacuo*. An analytical sample of 7 gave a negative Kaiser test.

A small sample of the resin **7** was treated with DBU/ mercaptoethanol in acetonitrile to release a mixture of **6a, 6c** and **6d** the relative quantities of these components being assessed by HPLC, measuring the peak areas at 254 and 386 nm (table).

A single bead of 7 was then selected and cleaved in the same way. The ratio of the components was again measured using HPLC.

Approximately 200 mg of resin **7** (0.056 μmol) was swollen with dichloromethane and the excess solvent drained away. Phenol (10.5 mg, 0.112 μmol) was added followed by 80% trifluoroacetic acid in dichloromethane such that this added volume constituted one third of the total. After agitating for two hours the pale red solvent was drained into a round-bottomed flask and the resin washed with distilled dichloromethane (3x3 ml). The combined organics were evaporated to dryness to afford a mixture of the primary amides **8a, 8c** and **8d**. The ratio of these components was assessed by measuring the magnitude of the integration of characteristic resonances in the ^{1}H nmr spectrum.

HPLC analysis (bulk beads)

	6a	**6c**	**6d**
Area ratio (254 nm)	**0.71**	**1.00**	**0.44**
Area ratio (386 nm)	**0.71**	**1.00**	**0.46**

HPLC analysis (single bead)

	6a	6c	6d
Area ratio (254 nm)	0.71	1.00	0.42
Area ratio (386 nm)	0.69	1.00	0.41

^{1}H nmr analysis

	8a	8c	8d
Ratio	0.74	1.00	0.42

The results set out in the tables above illustrate that there is a remarkably good correlation between the ratios obtained by the UV method and the ratios obtained by the well established and proven method of nmr analysis. Thus, the data demonstrate that UV detection and analysis can be used to provide a very accurate means of giving quantitative information about the reaction products of solid phase syntheses. Of particular significance is the fact that UV analysis has been shown to provide an accurate means of providing quantitative data even at the single bead level where the amounts of compound available for analysis are of the order of only 1 nanomole.

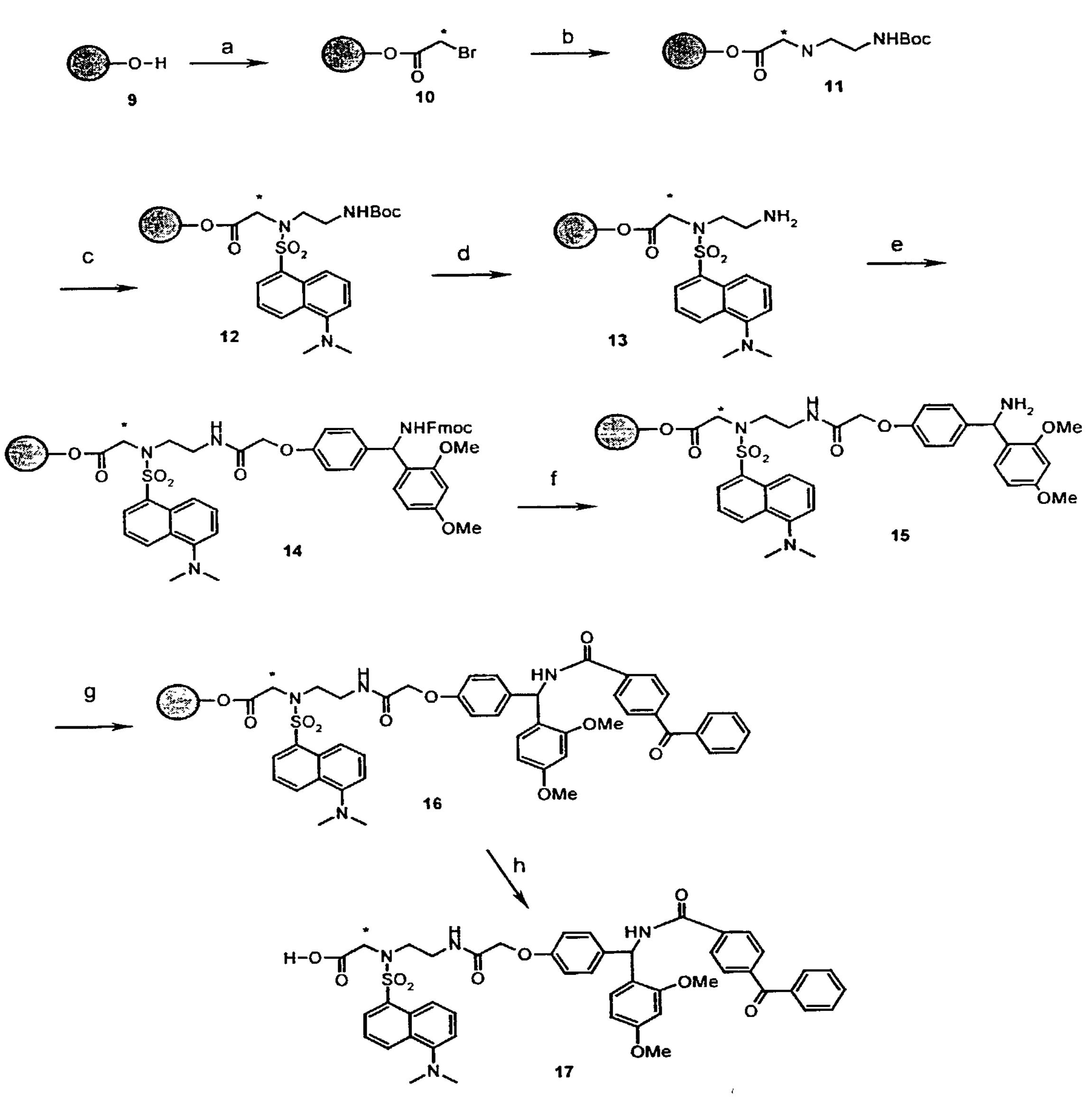
Furthermore, it can be seen from the example that the inclusion of a UV chromophore, and mass spectrometric sensitising group into the constructs enables rapid and simple qualitative and quantitative analysis of the products of a solid phase synthesis to be undertaken by the simple and well established procedures of HPLC in combination with UV detection and mass spectrometry.

EXAMPLE 2

Synthesis and Analysis of a Construct Containing a Dansyl Group as a UV Chromophore

A solid phase construct was formed on hydroxylated polystyrene resin by coupling together a base cleavable ester group, a diaminoethyl spacer group, an acid cleavable Rink linker and a dansyl group as shown in Scheme 4 below.

Scheme 4



2A. Preparation of Bromoacetylated Resin10

To a suspension of a hydroxylated polystyrene resin **9** (100-200 mesh) containing 1% DVB, and having a degree of substitution of 0.87 mmde/g (obtained from Novabiochem - batch 01.64.0110 (1g, loading 0.87 mmol = 1 eq.) in dry dimethylformamide (12ml) were added bromoacetic acid ^{12}C (0.6g, 5eq.), bromoacetic acid ^{13}C (0.65g, 5eq.), 4-

dimethylaminopyridine (DMAP) (catalytic amount), and di-isopropyl carbodiimide (DIC) (1.4ml, 10 eq.). The mixture was stirred for 24 hours at room temperature. The resin was filtered, washed with dimethylformamide (2 x 10ml), dichloromethane (5 x 10ml), methanol (2 x 10ml), dichloromethane again (2 x10 ml) and was then dried under reduced pressure. The presence of the bromoacetyl group on the resin was checked and confirmed by gel phase ^{13}C NMR spectroscopy.

Gel phase ^{13}C NMR (C_6D_6): 25.82 (1C);

2B. Preparation of Resin 11 Incorporating Diaminoethane Spacer Group

A suspension of resin **10** (0.95g, 0.83 mmol = 1 eq.) was formed in dry methylsulfoxide (10ml), N-Boc-diaminoethylene (1.3ml, 8.3 mmol = 10 eq.) was added, and the mixture was stirred at room temperature for 24 hours. The resulting resin **11** was filtered, washed with DMSO (2 x 10ml), dichloromethane (5 x 10ml), methanol (2 x 10 ml), dichloromethane (5 x 10 ml) and was then dried under reduced pressure. A chloranil test for secondary amine was positive.

Magic angle spinning (MAS) ^{1}H NMR (CD_2Cl_2): 5.06 (2H, bw) ; 3.41 (2H, bw) ; 3.14 (2H, m) ; 2.69 (2H, m) ; 1.42 (9H, s)

Elemental analysis (% nitrogen): %N theoretical = 2.38; %N experimental = 2.28

2C. Preparation of Resin 12 Incorporating a Dansyl Chromophore

To a suspension of the resin **11** (0.85g, 0.74 mmol = 1eq.) and dansyl chloride (2g, 1.48 mmol = 10eq.) in dry tetrahydrofuran (10ml) was added diisopropylethylamine (1.93ml, 11.1 mmol = 15 eq.) and the resulting mixture was stirred at room temperature for 24 hours. The resin **12** thus formed was filtered, washed with tetrahydrofuran (2 x 10 ml), dichloromethane (5 x 10ml), methanol (2 x 10 ml), dichloromethane (5 x 10ml) and dried under reduced pressure. A chloranil test carried out on **12** was negative confirming that all the secondary amine sites had been dansylated.

MAS ^{1}H NMR (CD_2Cl_2): 8.49 (1H, bs) ; 8.25 (1H, bs) ; 8.15 (1H, bs) ; 7.48 (2H,bm) ; 4.15 (2H, bs) ; 3.39 (2H, bs) ; 3.16 (2H, bs) ; 2.81 (6H, bs) ; 1.37 (9H, bs).

2D. Deprotection and Coupling of Resin 12 with Rink Linker to Give Resin 14

To a suspension of the resin **12** (0.74 mmol, 1 eq.) in dry dichloromethane (10ml) was added trifluoroacetic acid (2ml), and the mixture was stirred at room temperature for 24 hours.

The resulting resin **13** was filtered, washed with dichloromethane (5 x 10ml), methanol (2 x 10ml), dichloromethane (5 x 10ml) and dried under reduced pressure. A Kaiser test carried out to reveal the presence of primary amine was positive.

To a solution of Rink linker (3.9g, 10 eq.) in dry dimethylformamide (5ml) were added HOBT (0.97g, 10 eq.) and DIC (1.15ml, 10 eq.) and the resulting mixture was stirred at room temperature for 15 minutes before adding to the resin **13** in suspension in dimethylformamide (5ml) and diisopropylamine (0.250ml, 2 eq.). The mixture was stirred for 24 hours at room temperature. The resin **14** thus produced was filtered, washed with dimethylformamide (2 x 10ml), dichloromethane (5 x 10ml), methanol (2 x 10ml), dichloromethane (5 x 10ml) and dried under reduced pressure. Resin **14** gave a negative Kaiser test thereby confirming that the primary amino group of resin **13** had reacted completely with the Rink linker.

2E. <u>Deprotection of Resin 14 and Coupling with Substrate to Give Resin 16</u>

Resin **14** (49mg, 24.5μmol = 1 eq.) was suspended in a 20% piperidine in dimethylformamide solution (2ml) and the suspension was stirred for 2 hours. The resulting resin **15** was filtered, washed with dimethylformamide (2 x 10ml), dichloromethane (5 x 10ml), methanol (2 x 10ml), dichloromethane (5 x 10ml) and dried under reduced pressure. UV analysis to detect the Fmoc group revealed that the cleavage reaction to release the primary amine **15** had been quantitative. The presence of a primary amine group was confirmed by a positive Kaiser test.

To a solution of 4-benzoylbenzoic acid (55.4mg, 10 eq.) in dry dimethylformamide (0.5ml) were added HOBT (33mg, 10 eq.) and DIC (40μl, 10 eq.) and the resulting mixture was stirred at room temperature for 15 minutes before adding to the resin **15** in suspension in dimethylformamide (0.5ml). The mixture was stirred for 20 hours at room temperature. The resulting resin **16** was filtered, washed with dimethylformamide (2 x 10ml), dichloromethane (5 x 10ml), methanol (2 x 10ml), dichloromethane (5 x 10ml) and dried under reduced pressure. Resin **16** gave a negative Kaiser test thereby demonstrating that the acylation reaction had gone to completion and that no primary amine remained.

2F. <u>Cleavage of Construct Containing Dansyl Group and Substrate From the Resin</u>

To a suspension of resin **17** (45mg, loading estimated 0.5017mmol/g =>22.58μmol = 1 eq.) in tetrahydrofuran (0.4ml) was added a 1N sodium hydroxide solution (45μl, 2eq.) and the mixture was stirred at room temperature for 24 hours. The resin was filtered and washed with tetrahydrofuran and water. The filtrate was evaporated to give compound **17** in its sodium salt form..

^{1}H-NMR (DMSO) : 9.62 (1H, t) ; 9.28 (H, d) ; 8.39 (H, d) ; 8.33 (1H, d) ; 8.25(1H, d) ; 8.05 (2H, d) ; 7.8-7.65 (5H, m) ; 7.6-7.5 (4H, m) ; 7.34 (1H, d) ; 7.16 (1H, d) ; 7.07 (2H, d) ; 6.87 (2H, d) ; 6.6-6.5 (3H, m) ; 4.27 (2H, s) ; 3.74 (8H, s) ; 3.3 (2H, m) ; 3.25 (2H, m) ; 2.78 (6H, s).
MS-ES$^+$: 881-882 $[MNa]^+$ peak split present.

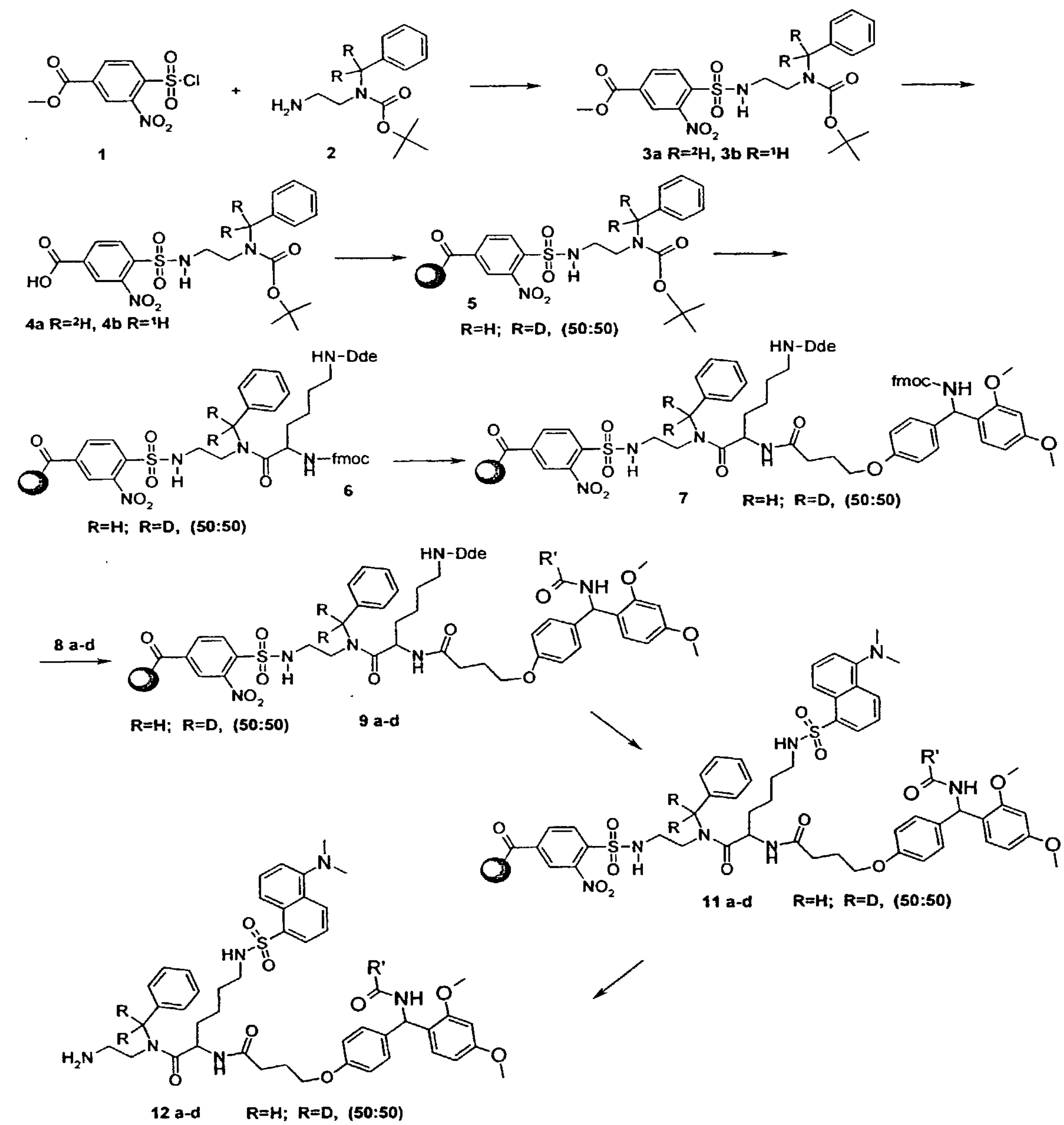
Analysis of the dansyl chromophore-containing compound **17** can be carried out in a similar manner to the method described in Examples 1F and 1G.

EXAMPLE 3

Preparation of a Second Construct Containing a Dansyl Group as a UV Chromophore

A second construct including a dansyl group as the chromophore was prepared. The sequence of steps leading to the construct is shown in Scheme 5 below. As can be seen from Scheme 5, the construct is analogous to the construct of Example 1 in that a sulphonamide linker group provides a first cleavage site to enable release of analytical fragment, and a Rink linker provides a second cleavage site to enable release of substrate. However, in this example, the chromophore, rather than being linked to the sulphonamide nitrogen, is attached to a lysine group positioned between the ethylene diamine chain and the Rink linker. .

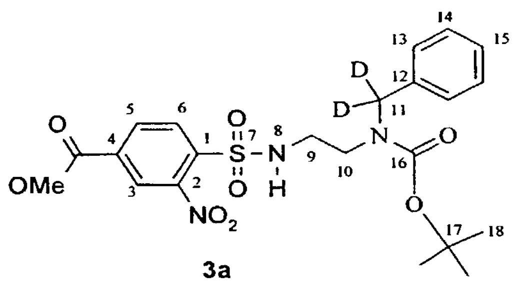
Scheme 5



Experimental for Scheme 5

In the following experimental details, the compound or construct numbers refer to the compounds and constructs identified in Scheme 5.

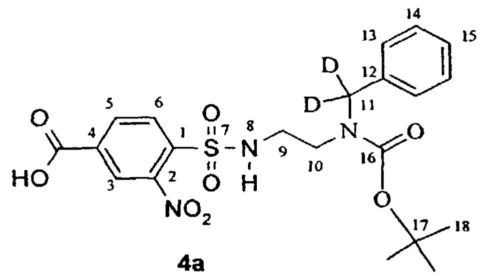
Preparation of Compound 3a



3a

To a stirred solution of 2-nitro-4-methylbenzoatesulfonyl chloride **1** (1.10 g, 3.90 mmol) and triethylamine (0.39 g, 3.90 mmol) in dry dichloromethane (40 ml), was added at 0°C the amine **2a** (1.00 g, 3.9 mmol) in dry dichloromethane (20 ml) over 30 min. The reaction mixture was stirred for a further 2 h at room temperature then concentrated *in vacuo* to give a yellow oil. Purification by chromatography with [MTBE:hexane (1:1)] as eluant followed by recrystallisation from MTBE (5 ml) and hexane (2 ml) furnished ***the sulfonamide 3a*** (1.53 g, 79%) as white flakes; mp 129-130°C; R_f 0.31 [MTBE:hexane (1:1)]; (Found: C, 53.2; H, 5.6; N, 8.5; S, 6.4. $C_{22}H_{25}D_2N_3O_8S$ requires C, 53.3; H, 5.5; N, 8.5; S, 6.5%); δ_H (DMSO-d^6) 1.40 (9H, s, H-18), 3.05 (2H, m, H-9), 3.20 (2H, dt, *J* 3 and 13, H-10), 3.9 (3H, s, O-Me), 7.10-7.35 (5H, m, H-13, 14 and 15), 8.09 (1H, d, *J* 8, H-6), 8.35 (1H, d, *J* 8, H-5), 8.40 (1H, m, H-8), 8.45 (1H, s, H-3),; δ_C (DMSO-d^6) 27.8 (C-18), 40.9 (C-9 and 10), 45.9 (C-11), 53.0 (O-Me), 79.1 (C-17), 124.8 (C-13), 127.0 (C-14 and 15), 128.3 (C-6), 130.0 (C-13), 132.9 (C-5), 134.2 (C-12), 136.4 (C-1), 147.5 (C-4), 154.7 (C-2), 163.5 (C-16 and C=O); MS (Electrospray +ve) m/z 496 $(MH)^+$; HPLC (Method A), R_t 6.07 min.

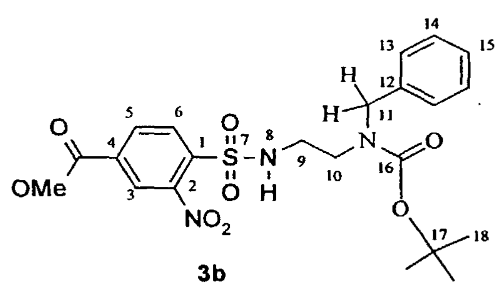
Preparation of Compound 4a



4a

The ester **3a** (0.40 g, 0.80 mmol) in methanol (2 ml) was treated with 1M aqueous sodium hydroxide (1.60 ml, 1.60 mmol) and stirred at room temperature for 1 hour. The reaction was then concentrated *in vacuo* and the residue dissolved in water (10 ml) then acidified with 1M hydrochloric acid (1.80 ml, 1.80 mmol) at 0°C. The resulting white precipitate was extracted with dichloromethane (3 x 10 ml). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure to ***the acid 4a*** (0.34 g, 88%) as a white solid; mp 143-145°C; R_F 0.58 [MTBE:hexane (1:1)]; δ_H (DMSO-d^6) 1.29 (9H, s, H-18), 3.02 (2H, m, H-9), 3.20 (2H, dt, *J* 3 and 13, H-10), 7.10-7.40 (5H, m, H-13, 14 and 15), 8.06 (1H, d, *J* 8, H-6), 8.28 (1H, s, H-3), 8.30 (1H, d, *J* 8, H-3); δ_C (DMSO-d^6) 27.9 (C-18), 40.9 (C-9 and 10), 45.9 (C-11), 79.1 (C-17), 124.5 (C-13), 127.1 (C-14 and 15), 128.4 (C-6), 129.5 (C-13), 132.5 (C-5), 139.2 (C-12), 147.3 (C-1), 155.1 (C-4), 164.8 (C-16 and C=O); (Found: MH^+, 482.156364 $C_{21}H_{23}D_2N_3O_8S$ requires MH+, 482.156615); HPLC (Method A), R_t 5.65 min.

Preparation of Compound 3b

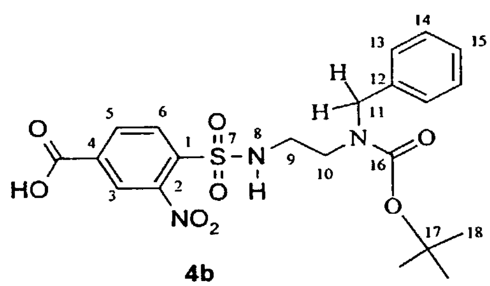


3b

To a stirred solution of 2-nitro-4-methylbenzoatesulfonyl chloride **1** (1.10 g, 3.90 mmol)

and triethylamine (0.39 g, 3.90 mmol) in dry dichloromethane (40 ml), was added at 0°C the amine **2b** (0.95 g, 3.9 mmol) in dry dichloromethane (20 ml) over 30 min. The reaction mixture was stirred for a further 3 h at room temperature then concentrated *in vacuo* to give a yellow oil. Purification by chromatography with [MTBE:hexane (1:1)] as eluant followed by recrystallisation from MTBE (5 ml) and hexane (2 ml) furnished ***the sulfonamide 3b*** (1.51 g, 79%) as white flakes; mp 131-132°C; R_f 0.28 [MTBE:hexane (1:1)]; (Found: C, 53.7; H, 5.4; N, 8.4; S, 6.5. $C_{22}H_{27}N_3O_8S$ requires C, 53.5; H, 5.5; N, 8.5; S, 6.5%); δ_H (DMSO-d^6) 1.38 (9H, s, H-18), 3.10 (2H, t, *J* 8, H-9), 3.24 (2H, t, *J* 8, H-10), 3.95 (3H, s, O-Me), 4.32 (2H, s, H11), 7.13-7.32 (5H, m, H-13, 14 and 15), 8.09 (1H, d, *J* 8, H-6), 8.29 (1H, s, H-3), 8.32 (1H, d, *J* 8, H-3); δ_C (DMSO-d^6) 28.3 (C-18), 40.2 (C-9 and 10), 46.2 (C-11), 53.2 (O-Me), 79.3 (C-17), 125.1 (C-13), 127.2 (C-14 and 15), 128.6 (C-6), 130.3 (C-13), 133.1 (C-5), 134.4 (C-12), 136.6 (C-1), 147.6 (C-4), 163.7 (C-16 and C=O); MS (Electrospray -ve) m/z 492 (M-H)$^-$; HPLC (Method A), R_t 5.99 min.

Preparation of Compound 4b

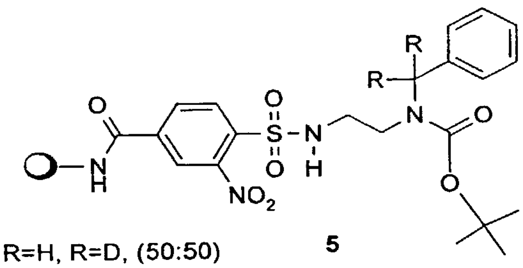


4b

The ester **3b** (0.40 g, 0.80 mmol) in methanol (2 ml) was treated with 1M aqueous sodium hydroxide (1.60 ml, 1.60 mmol) and stirred at room temperature for 1 hour. The reaction was then concentrated *in vacuo* and the residue dissolved in water (10 ml) then acidified with 1M hydrochloric acid (1.80 ml, 1.80 mmol) at 0°C. The resulting white precipitate was extracted with dichloromethane (3 x 10 ml). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure to ***the acid 4b*** (0.36 g, 93%) as a white solid; mp 123.7-124.8°C; R_F 0.59 [MTBE:hexane (1:1)]; δ_H (DMSO-d^6) 1.42 (9H, s, H-18), 3.12 (2H, m, H-9), 3.22 (2H, m, H-10), 4.38 (2H, s, H11), 7.18-7.42 (5H, m, H-13, 14 and 15), 8.11 (1H, d, *J* 8, H-6), 8.39 (1H, d, *J* 8, H-3); 8.45 (1H, s, H-3); δ_C (DMSO-d^6) 27.8 (C-18), 40.1 (C-9 and 10), 45.9 (C-11), 79.1 (C-17), 124.8 (C-13), 126.9 (C-14 and 15), 128.4 (C-6), 129.9 (C-13), 132.9 (C-5), 135.8 (C-12), 137.9 (C-1), 147.5 (C-4), 153.9 (C-2), 163.7 (C-16 and C=O); MS (Electrospray

-ve) m/z 478 $(M-H)^-$; HPLC (Method A), R_t 5.56 min.

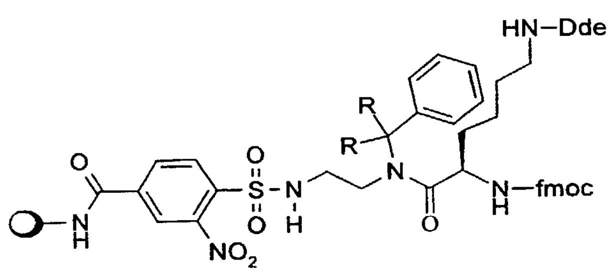
Preparation of Compound 5



R=H, R=D, (50:50) **5**

To a suspension of amine resin (Argogel-NH_2) (0.80 g, 0.32 mmol)), having a bead diameter of 150 μm and a loading of 0.4 mmol g^{-1}, in DMF (4 ml) and dichloromethane (4 ml) was added the benzoic acid **(4a)** (0.46 g, 0.96 mmol), and the benzoic acid **(4b)** (0.46 g, 0.96 mmol), followed by PyBOP (0.99 g, 1.92 mmol) and HOBT (0.26. g, 1.92 mmol) followed, 4 min. later by Hünig's base (0.49 g, 3.84 mmol). The reaction was agitated with nitrogen for 18 h and the resin then washed with dichloromethane (6 x 10 ml), DMF (6 x 10 ml), dichloromethane (6 x 10 ml), ether (2 x 10 ml) and dried *in vacuo* to give the ***sulfonamide resin 5*** (0.94 g, 97%) as a yellow solid; Kaiser test was negative; Bromophenol Blue test was negative.

Preparation of Compound 6

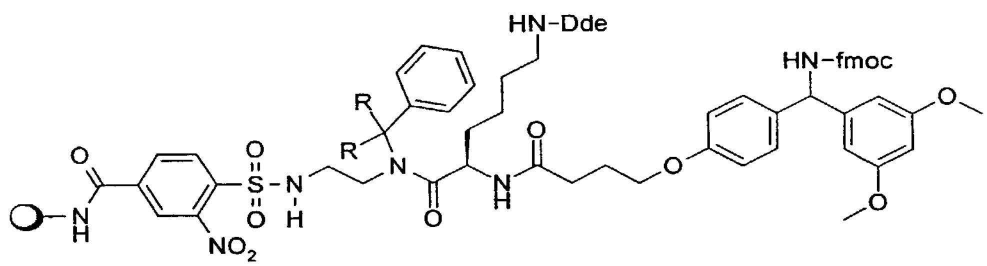


R=H; R=D, (50:50) **6**

The protected resin 5 (0.85 g, 0.34 mmol) was suspended in dichloromethane (2 ml) and treated with trifluoroacetic acid (2 x 4 ml) for 5 min. Solvents were removed by filtration and the resin washed with 10% DIPEA solution in DMF (3 x 5 ml), dichloromethane (6 x 5 ml), DMF (6

x 5 ml), dichloromethane (6 x 5 ml), ether (2 x 5 ml). The amine resin was then suspended in DMF (4 ml) and dichloromethane (2 ml) FMoc-Lys(Dde)-OH (0.86 g, 1.62 mmol) was added, followed by PyBOP (0.84 g, 1.62 mmol) and HOBT (0.22. g, 1.62 mmol). After 4 min. Hünig's base (0.42 g, 3.24 mmol) was added. The reaction was agitated with nitrogen for 18 h and the resin then washed with dichloromethane (6 x 10 ml), DMF (6 x 10 ml), dichloromethane (6 x 10 ml), ether (2 x 10 ml) and dried *in vacuo* to give the ***sulfonamide resin 6*** (0.96 g, 100%) as a yellow solid; Kaiser test was negative; Bromophenol Blue test was negative; Resin loading 99% as estimated by quantitative Fmoc group cleavage.

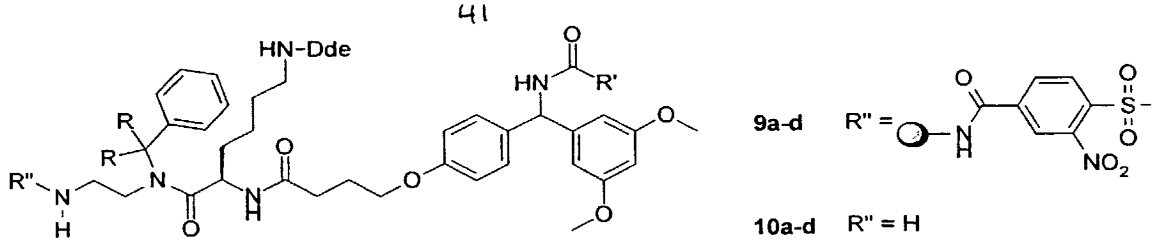
Preparation of Compound 7



R=H; R=D, (50:50) **7**

Resin **(6)** (0.93 g, 0.28 mmol) was treated with 20% Piperidine in DMF solution (2 x 5 ml) for 10 min. Solvents were removed by filtration and the resin washed with DMF (6 x 5 ml), dichloromethane (6 x 5 ml), and ether (2 x 4 ml). The resin was suspended in DMF (5 ml) and dichloromethane (5 ml) and the Rink acid **linker** (0.92 g, 1.62 mmol) was added, followed by PyBOP (0.84 g, 1.62 mmol) and HOBT (0.22 g, 1.62 mmol). After 4 min. Hunig's base (0.42g, 3.24 mmol) was added. The reaction was agitated with nitrogen for 3 h and the resin then washed with dichloromethane (6 x 5 ml), DMF (6 x 5 ml), dichloromethane (6 x 5 ml), ether (2 x 3 ml) and dried *in vacuo* to give the ***sulfonamide resin*** *7* (1.02 g, 98%) as a yellow solid; Kaiser test was negative; Bromophenol Blue test was negative; Resin loading 94% as estimated by quantitative Fmoc group cleavage.

Preparation of Compounds 9a-d and 10a-d



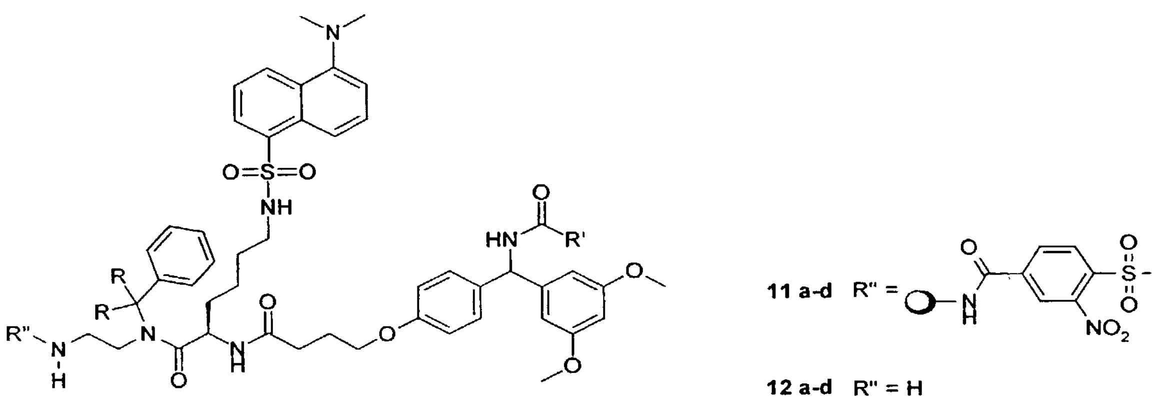
R=H; R=D, (50:50)

Resin **(7)** (70 mg, 0.02 mmol) was treated with 20% piperidine in DMF solution (2 x 1 ml) for 10 min. Solvents were removed by filtration and the resin washed with DMF (6 x 1 ml), dichloromethane (6 x 1 ml), ether (2 x 1 ml). The resin was suspended in DMF (0.5 ml) and dichloromethane (0.5 ml) and the appropriate acid **(8a-d)** (0.15 mmol) was added, followed by PyBOP (0.08 g, 0.15 mmol) and HOBT (0.02 g, 0.15 mmol). After 4 min. Hünig's base (0.04 g, 0.30 mmol) was added. The reaction was agitated with nitrogen for 3 h and the resin then washed with dichloromethane (6 x 1 ml), DMF (6 x 1 ml), dichloromethane (6 x 1 ml), ether (2 x 1 ml) and dried *in vacuo* to give the ***sulfonamide resins 9a-d*** as a yellow solid; Kaiser test and Bromophenol Blue test were negative in all four cases.

A small sample of each of the four resins was treated with DBU/Mercaptoethanol in acetonitrile to release ***10a-d*** which were analysed by low resolution mass spectrometry and RP HPLC (Method A):

10a (R'= Z-Gly-) MS (Electrospray +ve) m/z 961 and 963 $(MH)^+$; HPLC, R_t 4.01 min.
10b (R'= Boc-Abu-) MS (Electrospray +ve) m/z 955 and 957 $(MH)^+$; HPLC, R_t 4.09 min.
10c (R'= 1-naphthylacetoyl-) MS (Electrospray +ve) m/z 938 and 939 $(MH)^+$; HPLC, R_t 4.28 min.
10d (R'= Boc-Phe-) MS (Electrospray +ve) m/z 1017 and 1019 $(MH)^+$; HPLC, R_t 4.42 min.

Preparation of Compounds 11a-d and 12a-d



Resin **(9a-d)** (ca. 70 mg, 0.02 mmol) was treated with 10% hydrazine in DMF solution (3 x 1 ml) for 30 min. Solvents were removed by filtration and each resin washed with DMF (6 x 1 ml), dichloromethane (6 x 1 ml), ether (2 x 1 ml). To each resin portion was then added dansyl chloride (40 mg, 0.15 mmol) and triethylamine (15 mg, 0.15 mmol) in dichloromethane (1 ml). After 4 h reaction time the resins were washed with dichloromethane (6 x 1 ml), DMF (6 x 1 ml), dichloromethane (6 x 1 ml), ether (2 x 1 ml) and dried *in vacuo* to give the ***sulfonamide resins 11a-d*** as a yellow solid; Kaiser test and Bromophenol Blue test were positive in all four cases.

A small sample of each of the four resins was treated with DBU/Mercaptoethanol in acetonitrile to release ***12a-d*** which were analysed by low resolution mass spectrometry and RP HPLC (Method B):

12a (R'= Z-Gly-) MS (Electrospray +ve) m/z 1030 and 1032 $(MH)^+$; HPLC, R_t 4.77 min.
12b (R'= Boc-Abu-) MS (Electrospray +ve) m/z 1024 and 1026 $(MH)^+$; HPLC, R_t 4.94 min.
12c (R'= 1-naphthylacetoyl-) MS (Electrospray +ve) m/z 1007 and 1009 $(MH)^+$; HPLC, R_t 5.47 min.
12d (R'= Boc-Phe-) MS (Electrospray +ve) m/z 1086 and 1089 $(MH)^+$; HPLC, R_t 5.82 min.

It can be seen from the foregoing examples that the inclusion of a UV chromophore, and mass spectrometric sensitising group into the constructs enables rapid and simple

qualitative and quantitative analysis of the products of a solid phase synthesis to be undertaken by the simple and well established procedures of HPLC in combination with UV detection and mass spectrometry.

The foregoing examples are intended merely to be illustrative of the invention and are not intended to limit the invention in any way. On the contrary, it will readily be apparent that numerous modifications and alterations could be made to the constructs described in the examples above without departing from the principles underlying the invention and all such modifications and alterations are intended to be embraced by this application.

CLAIMS

1. A method of analysis of a solid phase construct; which method comprises:

(i) providing a chemical construct comprising a solid support Q having linked thereto via a connecting group Y a substrate R; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectrophotometry;

(ii) cleaving the connecting group Y at the first cleavage site to release the fragment Fr^u ; and

(iii) subjecting the fragment Fr^u to ultra violet, visible or fluorescence spectrophotometric analysis to quantify the substrate R.

2. A method according to claim 1 wherein the chromophore C^u has a principal log E_{max} value of at least 2.5.

3. A method according to claim 2 wherein the principal log E_{max} value is at least 1.5 times greater than the principal log E_{max} of the substrate R.

4. A method according to claim 2 or claim 3 wherein the chromophore C^u has a principal log E_{max} that is at least 2 times greater than the principal log E_{max} of the substrate R.

5. A method according to any one of the preceding claims wherein the chromophore C^u has an absorption band which is remote from any significant absorption bands due to the substrate R..

6. A method according to any one of the preceding claims wherein the spectrophotometric analysis is carried out at a wavelength corresponding to the principal E_{max} of the chromophore.

7. A method according to any one of claims 1 to 5 wherein the spectrophotometric analysis is carried out a wavelength corresponding to a non-principal absorption band of the chromophore.

8. A chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto via a connecting group Y a substrate R; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectroscopy, the chromophore C^u having a principal log E_{max} value of at least 2.5 and wherein (i) the principal log E_{max} value is at least 1.5 times greater than the principal log E_{max} of the substrate R; or (ii) , the chromophore C^u has an absorption peak at a wavelength remote from absorptions due to the substrate R..

9. A chemical construct according to claim 7 wherein the chromophore C^u has a principal log E_{max} which is at least 2 times greater than the principal log E_{max} of the substrate R.

10. A chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto via a connecting group Y a substrate R; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectroscopy, wherein the absorption characteristics of the chromophore C^u and the substrate R are such that at a given measurement wavelength, any errors in measurement of the quantity of substrate R (or any fragment or construct containing the fragment) arising from any overlap between absorption bands due to the chromophore and absorption bands due to the substrate R are less than 5%.

11. A chemical construct according to any one of claims 8 to 10 wherein the chromophore is a group containing a fused polycyclic aryl group.

12. A chemical construct according 11 wherein the polycylic aryl group is a C_6 - C_{30} polycyclic aryl group in which one or more (e.g. 1, 2, or 3) ring carbon atoms are optionally replaced by a heteroatom such as nitrogen, sulphur or oxygen.

13. A chemical construct according to claim 12 wherein the polycyclic aryl group is selected from polycyclic hydrocarbons such as naphthyl, phenanthrenyl and anthracenyl groups, and polycyclic heteroaryl groups such as acridine, or phenanthroline.

14. A chemical construct according to claim 13 wherein the chromophore contains an anthracenyl group.

15. A chemical construct according to claim 12 wherein the chromophore contains a dansyl (5-dimethylamino-1-naphthylsulphonyl) group.

16. A chemical construct according to any one of claims 8 to 15 wherein the fragment Fr^u contains a mass spectroscopic sensitising group G.

17. A chemical construct according to any one of claims 8 to 16 wherein the chemical fragment Fr^u contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

18. A chemical construct according to claim 16 wherein the means for imparting a characteristic signature to the mass spectrum of the fragment Fr^u is located between the first and second cleavage sites.

19. A chemical construct according to any one of claims 8 to 18 wherein the first and second cleavage sites are defined by first and second linker groups L^1 and L^2.

20. A chemical construct according to claim 19 wherein a spacer group A is interposed between the two linker groups L^1 and L^2, the spacer group A containing means for imparting a characteristic signature to the mass spectrum of the fragment Fr^u .

21. A chemical construct according to any one of claims 8 to 20 containing a sensitising group G which is a group which is ionisable under mass spectrometric conditions.

22. A chemical construct according to claim 21 wherein the group G is ionisable to form a positive ion under mass spectrometric conditions, for example electrospray mass spectrometric conditions.

23. A method of analysis of a solid phase construct; which method comprises:

(i) providing a chemical construct comprising a solid support Q having linked thereto via a connecting group Y a substrate R, the substrate R being present on each solid support in an amount of no more than 10 nanomoles; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectrophotometry;

(ii) isolating a solid support, or a plurality of solid supports not exceeding 20 in number;

(ii) treating the solid support(s) to cleave the connecting group at the first cleavage site to release the fragment Fr^u containing the substrate R; and

(Iii) subjecting the fragment Fr^u to ultra violet, visible or fluorescence spectrophotometric analysis to quantify the substrate R.

24. A method according to claim 23 wherein the solid support Q is a resin bead having an average diameter in the range from 90μm to 250μm.

25. A method according to claim 23 or claim 24 wherein the substrate R is present on each solid support in an amount of less than 5 nanomoles.

26. A method according to claim 25 wherein the substrate R is present on each solid support in an amount of less than 2 nanomoles.

27. A method according to any one of claims 23 to 26 wherein the number of solid supports isolated is less than 10, for example less than 5.

28. A method according to claim 27 wherein a single solid support is isolated and subjected to cleavage.

29. A method according to any one of claims 1 to 7 and 23 to 28 wherein the connecting group Y, chromophore C^u and fragment Fr^u are as defined in any one of claims 8 to 22.

30. A method of identifying a pharmaceutically useful substrate comprising preparing a library containing a plurality of chemical constructs as defined in any of claims 8 to 22, and subjecting the library to biological testing to identify biologically active substrates.

31. A method according to claim 30 which includes the further step of formulating a biologically active substrate thus identified with a pharmaceutically acceptable carrier to form a pharmaceutical composition.

32. An intermediate chemical construct for use preparing a chemical construct as defined in any one of claims 8 to 22, the intermediate construct having the formula Q-Y' wherein Y' is a reactive or protected form of the group Y.

33. An intermediate construct of the formula $Q\text{-}L^1\text{-}A^p$ wherein Q and L^1 are as defined in any one of claims 8 to 22 and A^p is a reactive or protected form of the spacer group A containing a chromophore C^u and optionally a peak splitting isotopic label.

34. An intermediate construct according to claim 33 having the general formula $Q\text{-}L^1\text{-}N(Alk - C^u)\text{-}Alk\text{-}NH\text{-}X^1$ wherein Alk is an alkylene group and X^1 is hydrogen or an aralkyl group.

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(54) Title: CHEMICAL CONSTRUCTS

(57) Abstract: A chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto via a connecting group Y a substrate R; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr^u comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectroscopy, the chromophore C^u having a principal log E_{max} value of at least 2.5 and wherein (i) the principal log E_{max} value is at least 1.5 times greater than the principal log E_{max} of the substrate R; or (ii) the chromophore C^u has an absorption peak at a wavelength remote from absorptions due to the substrate R; and to methods of analysis of products of solid phase synthesis using the constructs.

DECLARATION FOR "371" APPLICATION

COMBINED DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY	ATTORNEY'S DOCKET PI3750USW
	First Names Inventor: **CARR**
( ) Declaration submitted with initial filing or (X)Declaration submitted after initial filing (surcharge required 37CFR1.16(e))	***Complete if known:*** App No.: 10/089198
	Filing Date March 26, 2002
	Group Art Unit:

As below named inventor. I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CHEMICAL CONSTRUCTS

the specification of which (check only one item below):

[ ]is attached hereto.
OR
[X] was filed on ______________as United States application Serial No. __________________or **PCT** International

Application Number PCT/EP00/09639 filed 3 October 2000 and was amended on (MM/DD/YYYY) _________________(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

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Prior Foreign Application Number (s)	Country	Foreign Filing Date (MM/DD/YYYY))	PRIORITY CLAIMED
1. 9923577.2	GB	10/05/1999	x
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U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	PATENTED	PENDING	ABANDONED

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100				
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DECLARATION FOR "371" APPLICATION

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400	**INVENTOR'S SIGNATURE**			**DATE:**
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2	FULL NAME OF INVENTOR	FAMILY NAME ZARAMELLA	FIRST GIVEN NAME Allessio	SECOND GIVEN NAME/INITIAL
600	**INVENTOR'S SIGNATURE**			**DATE:**
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DECLARATION FOR "371" APPLICATION

COMBINED DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY	ATTORNEY'S DOCKET P13750USW
( ) Declaration submitted with initial filing or	First Names Inventor: **CARR**
(X)Declaration submitted after initial filing (surcharge required 37CFR1.16(e))	***Complete if known*:** App No.: 10/089198
	Filing Date March 26, 2002
	Group Art Unit:

As below named inventor. I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CHEMICAL CONSTRUCTS

the specification of which (check only one item below):

[ ]is attached hereto.

OR

[X] was filed on ______________as United States application Serial No. __________________or **PCT** International Application Number PCT/EP00/09639 filed 3 October 2000 and was amended on (MM/DD/YYYY) __________________(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56.

I hereby claim foreign priority benefits under 35, U.S.C. §119 (a)-(d) or §365(b) of any foreign applications(s) for patent or inventor's certificate or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

Prior Foreign Application Number (s)	Country	Foreign Filing Date (MM/DD/YYYY))	PRIORITY CLAIMED
1. 9923577.2	GB	10/05/1999	x
2.			
3.			
4.			
5.			

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date (MM/DD/YYYY)	
2.		
3.		
4.		
5.		

DECLARATION FOR "371" APPLICATION

COMBINED DECLARATION FOR UTILITY or DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY Continued	ATTORNEY'S DOCKET NUMBER **PI3750USW**

I hereby claim the benefit under 35, U.S.C. §120 of any United States application or §365(c) of any PCT international application designating the United States of America that is listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application.

PRIOR U.S. PARENT APPLICATION or PCT PARENT APPLICATION

		STATUS (Check one)		
U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	PATENTED	PENDING	ABANDONED

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith. (List name and registration number)

23347

PATENT TRADEMARK OFFICE

Send Correspondence to: 23347 PATENT TRADEMARK OFFICE	Direct Telephone Calls to: Frank P. Grassler 919-483-2482

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2	FULL NAME OF INVENTOR	FAMILY NAME **CARR**	FIRST GIVEN NAME **Robin**	SECOND GIVEN NAME/INITIAL **Arthur Ellis**
	INVENTOR'S SIGNATURE			**DATE:**
0	RESIDENCE & CITIZENSHIP	CITY **Cambridge**	STATE OR FOREIGN COUNTRY **UK**	COUNTRY OF CITIZENSHIP **GB**
1	POST OFFICE ADDRESS	POST OFFICE ADDRESS **250 Cambridge Science Park Milton Road**	CITY **Cambridge**	STATE & ZIP CODE/COUNTRY **London UK CB4 OWE**
2	FULL NAME OF INVENTOR	FAMILY NAME **GEHANNE**	FIRST GIVEN NAME **Sylvie**	SECOND GIVEN NAME/INITIAL
	INVENTOR'S SIGNATURE	Géhanne		**DATE:** 14/06/02
0	RESIDENCE & CITIZENSHIP	CITY **Verona**	STATE OR FOREIGN COUNTRY **IT**	COUNTRY OF CITIZENSHIP **FR**
2	POST OFFICE ADDRESS	POST OFFICE ADDRESS **GlaxoSmithKline Five Moore Drive, PO Box 13398**	CITY **Research Triangle Park**	STATE & ZIP CODE/COUNTRY **NC 27709 US**
2	FULL NAME OF INVENTOR	FAMILY NAME **KAY**	FIRST GIVEN NAME **Corinne**	SECOND GIVEN NAME/INITIAL
	INVENTOR'S SIGNATURE			**DATE:**
0	RESIDENCE & CITIZENSHIP	CITY **Cambridge**	STATE OR FOREIGN COUNTRY **UK**	COUNTRY OF CITIZENSHIP **FR**
3	POST OFFICE ADDRESS	POST OFFICE ADDRESS **Lensfield Road**	CITY **Cambridge**	STATE & ZIP CODE/COUNTRY **London CB2 1EW UK**

DECLARATION FOR "371" APPLICATION

2	FULL NAME OF INVENTOR	FAMILY NAME **PAIO**	FIRST GIVEN NAME **Alfredo**	SECOND GIVEN NAME/INITIAL
	INVENTOR'S SIGNATURE	[illegible]		**DATE:** 14/06/02
0	RESIDENCE & CITIZENSHIP	CITY **Verona**	STATE OR FOREIGN COUNTRY **IT**	COUNTRY OF CITIZENSHIP **IT**
4	POST OFFICE ADDRESS	POST OFFICE ADDRESS **GlaxoSmithKline Five Moore Drive, PO Box 13398**	CITY **Research Triangle Park**	STATE & ZIP CODE/COUNTRY **NC 27709 US**
2	FULL NAME OF INVENTOR	FAMILY NAME **WILLIAMS**	FIRST GIVEN NAME **Geoffrey**	SECOND GIVEN NAME/INITIAL **Martyn**
	INVENTOR'S SIGNATURE			**DATE:**
0	RESIDENCE & CITIZENSHIP	CITY **Birmingham**	STATE OR FOREIGN COUNTRY **GB**	COUNTRY OF CITIZENSHIP **NZ**
5	POST OFFICE ADDRESS	POST OFFICE ADDRESS **10 Holt Court South**	CITY **Birmingham**	STATE & ZIP CODE/COUNTRY **London B7 4EJ UK**
2	FULL NAME OF INVENTOR	FAMILY NAME **ZARAMELLA**	FIRST GIVEN NAME ~~**Allessio**~~ ALESSIO AZ 17.06.02	SECOND GIVEN NAME/INITIAL
	INVENTOR'S SIGNATURE	[illegible]		**DATE:** 17/06/02
0	RESIDENCE & CITIZENSHIP	CITY **Verona**	STATE OR FOREIGN COUNTRY **IT**	COUNTRY OF CITIZENSHIP **IT**
6	POST OFFICE ADDRESS	POST OFFICE ADDRESS **GlaxoSmithKline Five Moore Drive, PO Box 13398**	CITY **Research Triangle Park**	STATE & ZIP CODE/COUNTRY **NC 27709 US**

DECLARATION FOR "371" APPLICATION

COMBINED DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY	ATTORNEY'S DOCKET PI3750USW
	First Names Inventor: **CARR**
( ) Declaration submitted with initial filing or (X)Declaration submitted after initial filing (surcharge required 37CFR1.16(e))	***Complete if known:*** App No.: 10/089198
	Filing Date March 26, 2002
	Group Art Unit:

As below named inventor. I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CHEMICAL CONSTRUCTS

the specification of which (check only one item below):

[ ]is attached hereto.
OR
[X] was filed on ______________as United States application Serial No ____________________or **PCT** International

Application Number PCT/EP00/09639 filed 3 October 2000 and was amended on (MM/DD/YYYY) ______________(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56.

I hereby claim foreign priority benefits under 35, U.S.C. §119 (a)-(d) or §365(b) of any foreign applications(s) for patent or inventor's certificate or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

Prior Foreign Application Number (s)	Country	Foreign Filing Date (MM/DD/YYYY))	PRIORITY CLAIMED
1. 9923577.2	GB	10/05/1999	x
2.			
3.			
4.			
5.			

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date (MM/DD/YYYY)	
2.		
3.		
4.		
5.		

DECLARATION FOR "371" APPLICATION

COMBINED DECLARATION FOR UTILITY or DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY Continued	ATTORNEY'S DOCKET NUMBER **PI3750USW**

I hereby claim the benefit under 35, U.S.C. §120 of any United States application or §365(c) of any PCT international application designating the United States of America that is listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application.

PRIOR U.S. PARENT APPLICATION or PCT PARENT APPLICATION

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	STATUS (Check one) PATENTED	PENDING	ABANDONED

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith. (List name and registration number)

23347

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2	FULL NAME OF INVENTOR	FAMILY NAME **CARR**	FIRST GIVEN NAME **Robin**	SECOND GIVEN NAME/INITIAL **Arthur Ellis**
	INVENTOR'S SIGNATURE	Carr	Robin	DATE: 11/6/02.
0	RESIDENCE & CITIZENSHIP	CITY **Cambridge**	STATE OR FOREIGN COUNTRY **UK**	COUNTRY OF CITIZENSHIP **GB**
1	POST OFFICE ADDRESS	POST OFFICE ADDRESS **250 Cambridge Science Park Milton Road**	CITY **Cambridge**	STATE & ZIP CODE/COUNTRY **London UK CB4 OWE**
2	FULL NAME OF INVENTOR	FAMILY NAME **GEHANNE**	FIRST GIVEN NAME **Sylvie**	SECOND GIVEN NAME/INITIAL
	INVENTOR'S SIGNATURE			DATE:
0	RESIDENCE & CITIZENSHIP	CITY **Verona**	STATE OR FOREIGN COUNTRY **IT**	COUNTRY OF CITIZENSHIP **FR**
2	POST OFFICE ADDRESS	POST OFFICE ADDRESS **GlaxoSmithKline Five Moore Drive, PO Box 13398**	CITY **Research Triangle Park**	STATE & ZIP CODE/COUNTRY **NC 27709 US**
2	FULL NAME OF INVENTOR	FAMILY NAME **KAY**	FIRST GIVEN NAME **Corinne**	SECOND GIVEN NAME/INITIAL
	INVENTOR'S SIGNATURE			DATE:
0	RESIDENCE & CITIZENSHIP	CITY **Cambridge**	STATE OR FOREIGN COUNTRY **UK**	COUNTRY OF CITIZENSHIP **FR**
3	POST OFFICE ADDRESS	POST OFFICE ADDRESS **Lensfield Road**	CITY **Cambridge**	STATE & ZIP CODE/COUNTRY **London CB2 1EW UK**

DECLARATION FOR "371" APPLICATION

2	FULL NAME OF INVENTOR	FAMILY NAME **PAIO**	FIRST GIVEN NAME **Alfredo**	SECOND GIVEN NAME/INITIAL
	INVENTOR'S SIGNATURE			**DATE:**
0	RESIDENCE & CITIZENSHIP	CITY **Verona**	STATE OR FOREIGN COUNTRY **IT**	COUNTRY OF CITIZENSHIP **IT**
4	POST OFFICE ADDRESS	POST OFFICE ADDRESS **GlaxoSmithKline Five Moore Drive, PO Box 13398**	CITY **Research Triangle Park**	STATE & ZIP CODE/COUNTRY **NC 27709 US**
2	FULL NAME OF INVENTOR	FAMILY NAME **WILLIAMS**	FIRST GIVEN NAME **Geoffrey**	SECOND GIVEN NAME/INITIAL **Martyn**
	INVENTOR'S SIGNATURE			**DATE:**
0	RESIDENCE & CITIZENSHIP	CITY **Birmingham**	STATE OR FOREIGN COUNTRY **GB**	COUNTRY OF CITIZENSHIP **NZ**
5	POST OFFICE ADDRESS	POST OFFICE ADDRESS **10 Holt Court South**	CITY **Birmingham**	STATE & ZIP CODE/COUNTRY **London B7 4EJ UK**
2	FULL NAME OF INVENTOR	FAMILY NAME **ZARAMELLA**	FIRST GIVEN NAME **Allessio**	SECOND GIVEN NAME/INITIAL
	INVENTOR'S SIGNATURE			**DATE:**
0	RESIDENCE & CITIZENSHIP	CITY **Verona**	STATE OR FOREIGN COUNTRY **IT**	COUNTRY OF CITIZENSHIP **IT**
6	POST OFFICE ADDRESS	POST OFFICE ADDRESS **GlaxoSmithKline Five Moore Drive, PO Box 13398**	CITY **Research Triangle Park**	STATE & ZIP CODE/COUNTRY **NC 27709 US**

DECLARATION FOR "371" APPLICATION

COMBINED DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY

ATTORNEY'S DOCKET	PI3750USW
First Names Inventor	CARR
Complete if known: App No.:	
Filing Date	March 26, 2002
Group Art Unit:	

( ) Declaration submitted with initial filing or

(X)Declaration submitted after initial filing (surcharge required 37CFR1.16(e))

As below named inventor. I hereby declare that:

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CHEMICAL CONSTRUCTS

the specification of which (check only one item below):

[ ]is attached hereto.
OR
[X] was filed on ______________as United States application Serial No. __________________or **PCT** International

Application Number PCT/EP00/09639 filed 3 October 2000 and was amended on (MM/DD/YYYY) ________________(if applicable)

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PRIOR FOREIGN AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

Prior Foreign Application Number (s)	Country	Foreign Filing Date (MM/DD/YYYY))	PRIORITY CLAIMED
1. 9923577.2	GB	10/05/1999	x
2.			
3.			
4.			
5.			

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date (MM/DD/YYYY)	
2.		
3.		
4.		
5.		

DECLARATION FOR "371" APPLICATION

COMBINED DECLARATION FOR UTILITY or DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY Continued

ATTORNEY'S DOCKET NUMBER
P13750USW

I hereby claim the benefit under 35, U.S.C. §120 of any United States application or §365(c) of any PCT international application designating the United States of America that is listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. PARENT APPLICATION or PCT PARENT APPLICATION

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	STATUS (Check one) PATENTED	PENDING	ABANDONED

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith. (List name and registration number)

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PATENT TRADEMARK OFFICE

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Direct Telephone Calls to:
Frank P. Grassler
919-483-2482

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2	FULL NAME OF INVENTOR	FAMILY NAME: CARR \ oO	FIRST GIVEN NAME: Robin	SECOND GIVEN NAME/INITIAL: Arthur Ellis
	INVENTOR'S SIGNATURE			DATE:
0	RESIDENCE & CITIZENSHIP	CITY: Cambridge GBX	STATE OR FOREIGN COUNTRY: UK	COUNTRY OF CITIZENSHIP: GB
1	POST OFFICE ADDRESS	POST OFFICE ADDRESS: 250 Cambridge Science Park Milton Road	CITY: Cambridge	STATE & ZIP CODE/COUNTRY: London UK CB4 OWE
2	FULL NAME OF INVENTOR	FAMILY NAME: GEHANNE	FIRST GIVEN NAME: Sylvie	SECOND GIVEN NAME/INITIAL:
	INVENTOR'S SIGNATURE			DATE:
0	RESIDENCE & CITIZENSHIP	CITY: Verona ITX	STATE OR FOREIGN COUNTRY: IT ITX	COUNTRY OF CITIZENSHIP: FR
2	POST OFFICE ADDRESS	POST OFFICE ADDRESS: GlaxoSmithKline Five Moore Drive, PO Box 13398	CITY: Research Triangle Park	STATE & ZIP CODE/COUNTRY: NC 27709 US
2	FULL NAME OF INVENTOR	FAMILY NAME: KAY	FIRST GIVEN NAME: Corinne	SECOND GIVEN NAME/INITIAL:
	INVENTOR'S SIGNATURE	[signature]		DATE: 26th July 2002
0	RESIDENCE & CITIZENSHIP	CITY: Newhouse GBX	STATE OR FOREIGN COUNTRY: UK	COUNTRY OF CITIZENSHIP: FR
3	POST OFFICE ADDRESS	POST OFFICE ADDRESS: Organon Laboratories	CITY: Newhouse, Lanarkshire	STATE & ZIP CODE/COUNTRY: Scotland ML1 5SH UK

DECLARATION FOR "371" APPLICATION

2	FULL NAME OF INVENTOR	FAMILY NAME PAIO 4.00	FIRST GIVEN NAME Alfredo	SECOND GIVEN NAME/INITIAL
	INVENTOR'S SIGNATURE			DATE:
0	RESIDENCE & CITIZENSHIP	CITY Verona ITX	STATE OR FOREIGN COUNTRY IT	COUNTRY OF CITIZENSHIP IT
4	POST OFFICE ADDRESS	POST OFFICE ADDRESS GlaxoSmithKline Five Moore Drive, PO Box 13398	CITY Research Triangle Park	STATE & ZIP CODE/COUNTRY NC 27709 US
2	FULL NAME OF INVENTOR	FAMILY NAME WILLIAMS 5.00	FIRST GIVEN NAME Geoffrey	SECOND GIVEN NAME/INITIAL Martyn
	INVENTOR'S SIGNATURE			DATE:
0	RESIDENCE & CITIZENSHIP	CITY Birmingham GBH	STATE OR FOREIGN COUNTRY GB	COUNTRY OF CITIZENSHIP NZ
5	POST OFFICE ADDRESS	POST OFFICE ADDRESS 10 Holt Court South	CITY Birmingham	STATE & ZIP CODE/COUNTRY London B7 4EJ UK
2	FULL NAME OF INVENTOR	FAMILY NAME ZARAMELLA 6.00	FIRST GIVEN NAME Allessio	SECOND GIVEN NAME/INITIAL
	INVENTOR'S SIGNATURE			DATE:
0	RESIDENCE & CITIZENSHIP	CITY Verona ITX	STATE OR FOREIGN COUNTRY IT	COUNTRY OF CITIZENSHIP IT
6	POST OFFICE ADDRESS	POST OFFICE ADDRESS GlaxoSmithKline Five Moore Drive, PO Box 13398	CITY Research Triangle Park	STATE & ZIP CODE/COUNTRY NC 27709 US